Introduction

1 Background:

Paramphistomum is one of the common parasites in the rumen and reticulum of sheep, goats, cattle and water buffaloes. Light infection doesn't cause serious damage to the animals, but massive number of immature *Paramphistomum* can migrate through intestinal tract causing acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young animals. *Paramphistomum* in duodenum and ileum are plug feeders and cause haemorrhage which leads to bleeding and diarrhea and bleeding for prolonged period may cause anaemia, which further weaken the host. Mature *Paramphistomum* are also responsible for ruminitis irregular rumination, unthrifitiness, lower nutrition conversion and loss of body condition, decrease in milk production and reduction of fertility (*Mogdy et al 2009*) Paramphistomiasis is worldwide in distributed, but the highest prevalence has been reported in tropical and sub tropica regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia. The epidemiology of *Paramphistomum* is determined by several factors governed by parasite-host-environment interactions. The major epidemiological variable influencing worm burdens of animals is the infection rate from pastures It is also influenced by the climatic requirement for egg hatching, development and survival of the larvae in pasture (*Melaku et al., 2012*)

Acute paramphistomosis is caused by massive infection with immature worms in the small intestine. They attach themselves to the intestinal mucosa, drawing pieces of the mucosa into their suckers causing

strangulation, necrosis and haemorrhage. Acute paramphistomosis usually occurs in young cattle less than two years of age and is charactarised by listlessness and anorexia. Profuse diarrhoea (which can sometimes be projectile) develops two-four weeks after infection. The feces are very fluid and may even contain immature flukes. Sub-mandibular oedema has been noted in several outbreaks and anaemia has also frequently been described. The association between the presence of adult flukes in the rumen and clinical disease has not been well established, although the presence of the parasite is often complicated by other concomitant conditions (associated with animals in poor condition, ill thrift and other parasitic diseases) (De Waal, 2011). There is little evidence regarding the pathogenesis of adult flukes to their hosts, but severe damage to the mucosa of the rumen was reported in heavy infection in experimentally infected sheep (*Eslami et al., 2011).*

2 Synonyms:

Paramphistoma amphistomiasis

3 History:

The first report of the causative agent of paramphistomatosis of cattle was in the coastal region of Algeria is described. On the basis of histological finding, causative agent was found to be a Paramphistomum daubneyi, Lymnaea truncatula was found to be present as a potential intermediate host at the localities where the cattle harboring these trematodes were kept. It was suggested that high intensity of invasion by the trematodes (up to 2204

specimens in one host) caused severe helminthosis in some cases. The finding of the causative agent of bovine paramphistomatosis in Algeria draws attention to the need of further investigation of trematodosis in cattle kept in that country(*Pacenovský et al., 1987*).

4 LIFE CYCLE:

Paramphistomum has an indirect life cycle with fresh water snails as the intermediate hosts, e.g. the genus *Bulinus, Planorbis, Stagnicola,* These snails are found in permanent and temporary watercourses, irrigation channels, swamps, dam edges and depressions, they are normally found attached to vegetation in these habitats (*NSW DPI 2007*). Adult flukes in the stomach lay eggs that are shed outside with the feces. About 2 weeks later miracidia hatch out of the eggs. They swim in the water until they find a suitable snail. They penetrate into the snail and continue development to sporocysts and rediae, which can multiply asexually and produce daughter rediae. Each redia produces several cercariae, the next developmental stage. Out of a single miracidium up to 30 cercariae can develop. Cercariae abandon the snail, swim around and attach to the vegetation where they encyst and become metacercariae, which are infective for final hosts that feed on infested vegetation. Encysted metacercariae do not survive dryness, but can survive and remain infective for up to 1 year in a humid and temperate environment, and are capable of overwintering (*parasitepedia 2013*).

Livestock ingests metacercariae while grazing in contaminated pastures. Once in the small intestine the young flukes leave the cysts, attach to the

intestinal mucosa and continue development. They feed on the tissues of the gut wall. Later on they detach from the gut's wall and migrate to the rumen, where they complete development to adult flukes and start producing eggs. After ingestion by the final host it takes 2 to 4 months for metacercariae to complete development and start laying eggs (pre-patent period) (*parasitepedia 2013*).

Figure 1 : Planorbid snails, the intermediate host for stomach fluke (NSW DPI 2007)

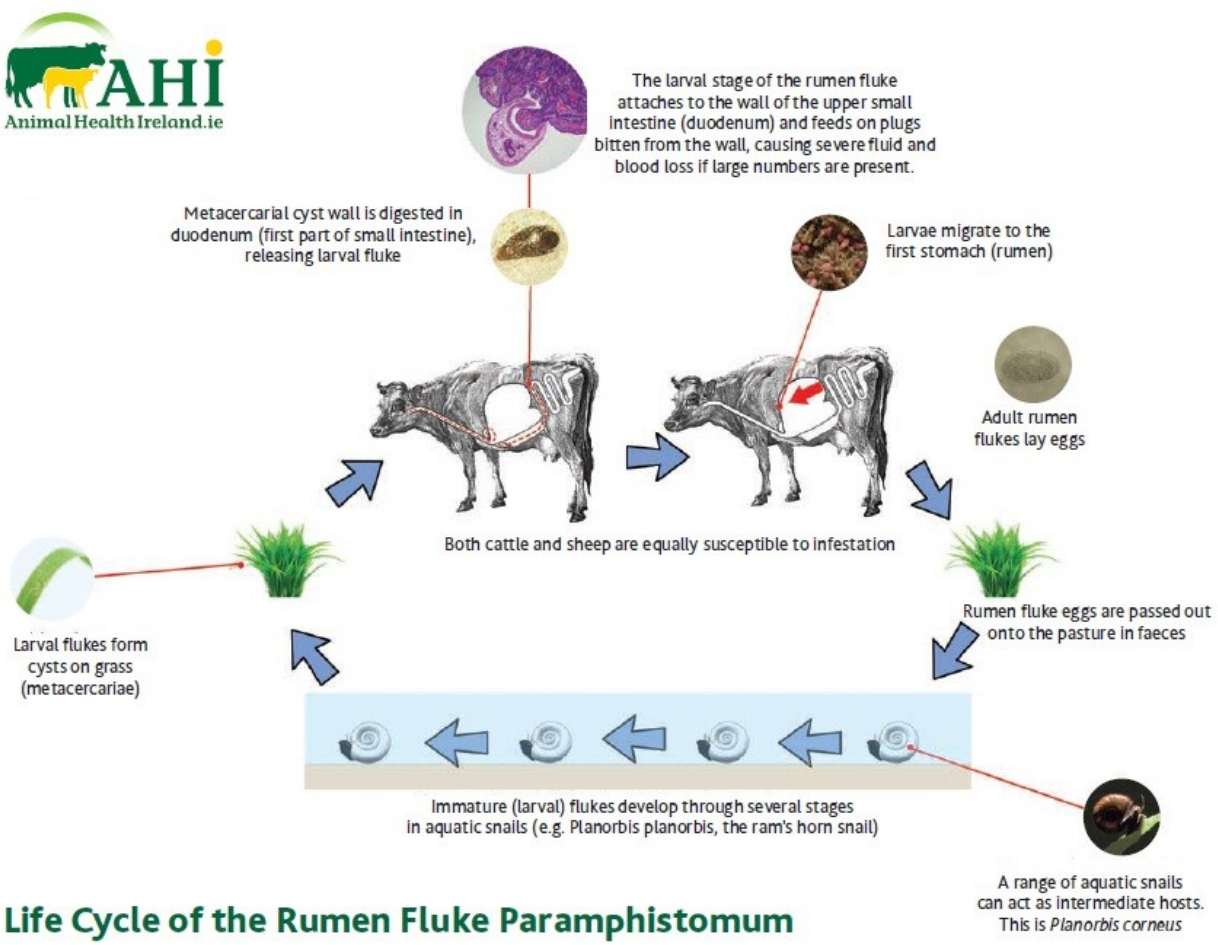

Figure 2 : life cycle of paramphistomum (*parasitepedia 2013*)

5 Justification :

paramphistoma infestation is an important disease but neglected and it is a public health problem in Africa, especially in rural communities . In rabak, *paramphistoma* may be one of the major infectious diseases becuase most abattoirs in rabak are not well qualified , and sheep, cattle and goats are still slaughtered traditionally . Pramphistoma is considered a

major public health problem in Sudan . Many animals are infected with pramphistoma. Determination of the prevalence of the disease in rabak is very important in order to explore the size of the problem which helps to control the disease. Paramphistomiasis infection are thought to be associated with the presence or absence of intermediate snail habitats in the grazing areas of the animals.

Since tropical paramphistoma is a significant factor in limiting livestock production, the development of sustainable strategies for controlling paramphistoma infection is a priority. Strategic use of anthelmintics, enhancement of host resistance by genetic improvement or by the use of vaccines, biological control and better herd management all have a role to play in sustainable control of paramphistoma .

6. objectives:

The objectives of this study were:

1/ To estimate the prevalence of bovine paramphistomiasis in Rabak.

2/ To investigate the potential risk factors associated with the disease.

Chapter One

Literature Review

1.1 Classification:

According to *Zeder (1790) paramphistoma* was classified as follows:

Kingdom: Animalia

Phylum: Platyhelminthes

Class: trematoda

Subclass: digenea

Order: Echinostomida

Family: paramphistomatidae

Genus: paramphistomum
Cotylophoron
Calicophoron
Explanatum
Gigantocotyle
Ugandocycle

Type species: P.cervi

P.cotylophorum

P.microbothrium

P.gotoi

P.grande

P.hiberniae

P.ichikawai

P.epiclitum

1.2 ***Etiology :***

Amphistomiasis in farm and wild mammals is due to infection of paramphistomes, such as the species of Paramphistomum, Calicophoron, Cotylophoron, Pseudophisthodiscus,et

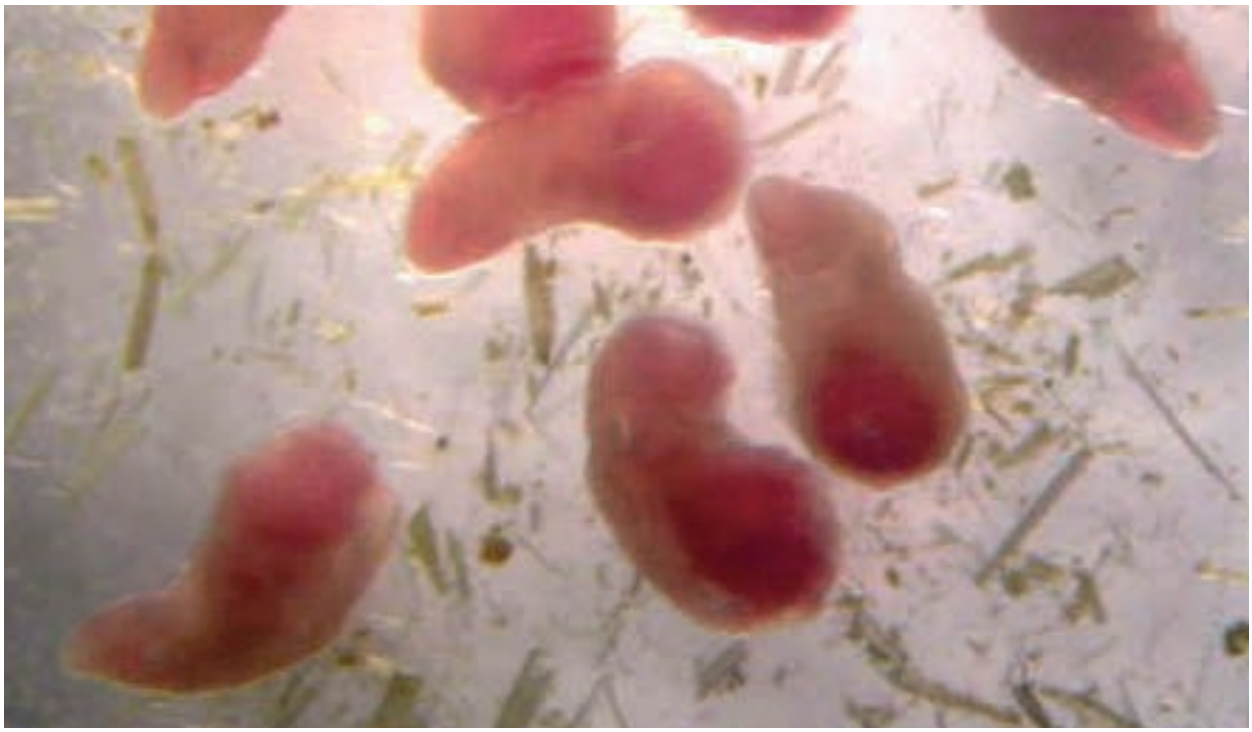

Figure3: Mature *Paramphistomum sp (*Sanabria et al 2008*)*

These are essentially rumen flukes, of which Paramphistomum cervi is the most notorious in terms of prevalence and pathogenicity. Infection occurs through ingestion of contaminated vegetables and raw meat, in which the viable infective metacercaria are reported from snails, which are the intermediate hosts (*chai et al.,2009*) The immature flukes are responsible for destroying the mucosal walls of the alimentary tract on their way to growing into adults. It is by this fervent tissue obliteration and appearance of clinical symptoms are manifested. The adult flukes, on the other hand, are quite harmless, as they merely prepare for reproduction (*brown et al.,2005*)

1.3 Description of paramphistoma warm :

The generic name (Greek: *para* meaning "similar" [to *Amphistoma*], *amphi* meaning "on both sides", and *stoma* for "mouth") is given due to the presence of an anterior oral sucker and a posterior larger ventral sucker in adult worms (Boray 1959) . The body is minute, measuring less than a centimetre. The body is covered with a highly folded tegument, which in turn is provided with sensory papillae. *Paramphistomum* are all hermaphrodite, having both male and female reproductive systems in the posterior region of the body (*Olsen, 1974*).

1.4 Clinical signs

Most livestock have only light stomach fluke infections. They show no signs of disease due to presence of adult flukes or small numbers of immature fluke. Heavy infections with the immature fluke may cause decreased appetite, listlessness and weight loss. Fluid, foul-smelling diarrhoea, dehydration and may terminate in death of the animal. Moderate infections with the immature fluke may cause reduced weight gains or milk production, or ill-thrift. Immature fluke live in the small intestine of ruminants where they attach themselves to the intestinal mucosa with powerful suckers. In large numbers, they destroy part of the mucosa and cause acute inflammation of the intestine. Death may occur in severe infections (*NSW 2007*).

Figure 4 Calf scouring due to stomach fluke infection. Note emaciated condition of animal (NSW 2007)

1.5 Diagnosis:

Provisional diagnosis is usually made on history and clinical signs of the disease (anorexia, polydipsia and projectile diarrhoea) and the presence of immature paramphistomes in the fluid faeces or at post mortem examination. Faecal examination for eggs at this stage is usually unrewarding as the disease is in the prepatent phase. Immature flukes are conical, pink in colour and 1-5mm long. The faecal sedimentation technique , commonly used for Fasciola diagnosis is the most suitable for identify the eggs in faeces. The eggs are oval and operculate, resembling that of *F. hepatica*; however, they are slightly larger and clear (transparent) rather than yellow in colour. The addition of a contrast stain such as methylene blue may help to differentiate these two species of eggs. The adult flukes are pear-shaped and red in colour, approximately 1cm long with a sucker at the tip of the cone and another sucker ventrally at the posterior-end (*Waal, 2011).*

ELISA is being practiced as the most effective diagnostic technique for detection of anti-parasitic antibodies (*Shabih et al., 2006).* Indirect plate enzyme-linked immunosorbent assay was standardized and evaluated for its effecteffness in immunodiagnosis of paramphistomosis in experimental and clinical cases in sheep, goat, cattle and buffaloes by using somatic whole adult antigen of *Paramphistomum epiclitum* and *Gastrothylax crumenifer*. Plate enzymelinked immunosorbent assay (ELISA) was standardized using 2 µg/ml of antigen concentration with 1:200 and 1:1,000 of sera and conjugate dilution. Indirect Plate ELISA was able to demonstrate the antibody titre at different weeks postinfection in experimental sheep. Immune response at weekly interval varied in all the group of experimental sheep (*Kaur et al., 2009*)

1.6 Postmortem:

At post mortem there is amarked haemorrhagic enteritis with large numbers of the immature worm parasites on the mucosa or contents of the duodenum and upper ileum, subcutaneous oedema, gelatinous fatty degeneration. Extensive catarrhal or haemorrhagic duodenitis or jejunitis with destruction of associated glands and lymph nodes are the main histopathological features. Immature flukes may be found embedded in the duodenal mucosa. There is amarked fall in total plasma protiens due to increased leakage of plasma albumin (*Kusiluka et al., 1996*).

1.7 Treatment :

Most drugs licensed for the treatment of fasciolosis are not effective against paramphistomosis. Only a few drugs have been shown to have an efficacy against either the immature and/or mature flukes namely; niclosamide, oxyclozanide, rafoxinide and resorantel. However, since paramphistomosis is seldom a significant economic problem in many temperate climates, these drugs are often not available and/or lienced for the treatment of paramphistomes. In Ireland, oxyclozanide is the only practical option for the treatment *(wall., 2011*)

1.8 Prevention and control:

Where rumen flukes are endemic, preventive measures are a must to reduce the snail populations, the infection of pastures with infective stages, or the access to livestock to highly infested pastures. Vector snails are aquatic and live in water (e.g. streams, lakes, pools, swamps, marshes, irrigation channels, ditches, ponds, watering holes, waterlogging, etc.) and are enormously prolific. Whatever measures help keeping the pastures dry are encouraged, either to reduce the snail population, or to shorten the survival time of encysted metacercariae, e.g.:

- Ensuring an adequate drainage
- Building watering points on solid ground, without puddles
- Make unavoidable ditches or channels less attractive to the snails by: making the borders steeper and/or cover them with concrete to eliminate the surrounding vegetation, drying them completely out periodically, etc.

- eliminate small water points that support the snails, e.g. hardened footprints (of shoes or car tires).

If permanent humid environments cannot be eliminated, they have to be fenced to prevent livestock from grazing there.Livestock infected with rumen flukes can develop a certain level of natural immunity that will make them more resistant to massive attacks by young flukes. Keeping livestock healthy and well fed diminishes the harm caused by rumen flukes and favors the development of the previously mentioned natural immunity. There are so far no vaccines against rumen flukes. Biological control of rumen flukes (i.e. using their natural enemies) is so not Feasible.*(parasitepedia.,2013)*

1.9 Epidemiology:

Flooding, caused by heavy rains, results in the dispersal of snails from permanent water masses, such as lakes and ponds. Paramphistome eggs, deposited in these areas by grazing animals, hatch and infect the snails. Outbreaks of disease generally occur in the dry months of the year when the receding water uncovers herbage contaminated with encysted metacercariae in these areas. In the UK, it has been suggested that dispersal of snails by flooding events and changes in farm-management practices may be responsible for the apparent emergence of the parasite (*Foster et al., 2008*).

Previous infection and the age of the host animal afford some protection against reinfection. Acute disease is usually seen in young animal less than two years of age, older (adult) animals often continue to harbour for snails Sheep appear susceptible throughout their lives and multiple infections only result in partial immunity to reinfection.(*Waal, 2011*).

1.9.1 Geographic distribution:

Paramphistoma is considered as worldwide in prevalence. It is most commonly found in tropical and subtropical regions, including Australia, Asia, Africa, Easter Europe, and Russia. The most debilitating cases are reported in Europe from Bulgaria, Italy, France, and Poland and also in Asia from Thailand, India, and China. The parasitic infection was first described from Punjab, India (*Boray., 1959*).

1.9.2 Previous Studies:

A study was carried out to determine the prevalence and intensity of paramphistomiasis in native sheep from Mazanderan province, in the north of Iran in association with sex, age, breed and season. During the 4 seasons of 2008, at meat inspection the rumen and reticulum of 132 native cattle and 104 mixed breed were examined by naked eye for paramphistomiasis. the result obtained showed overall prevalence rate and Mean SE of intensity 33.9% paramphistomes per animal, 40.9% in sheep, and 25% in mixed breeds, respectively. A few paramphistomes were collected from the reticulum of a native sheep. There was no significant relation between the intensity of the infection and breed (P=0.094), and age and the infection (P=0.016) were significant. The older group (5≤) harbored more trematodes than ≤2 and 3–4-year-old, and p-values: P=0.026 and P=0.032 were significant, respectively. (*Eslami et al.,2011*).

A study was conducted to investigate the prevalence of parasitic diseases in different abattoirs in selective area of Bangladesh. Animals were examined for post-mortem changes in different abattoirs of those districts.

The study started from February, 2008 to August, 2008. The total number of animals examined were 3510, among them 1460 cattle, 620 buffaloes, 970 goats and 460 sheep. Age, sex and breed of the examined animals were recorded . The overall prevalence of hydatidosis was highest (26.01%) followed by fascioliasis (20.74%), and amphistomiasis (19.62%). The prevalence of the above mentioned diseases was higher in older animals. The prevalence of hydatidosis, fascioliasis and amphistomiasis was higher in male in cattle and goats, but the prevalence of those diseases was distinctly higher in female animals buffaloes and sheep. The proportional prevalence of different disease conditions in cattle was much higher in Hariana breed than those of local and crossbred cattle.(*Hazza et al 2010*)

A retrospective study was carried out over a 10- to 12-years period to analyze the changes in prevalence of natural fasciolosis and paramphistomosis among cattle and snails in central France, and to determine the causes which had induced these changes. The prevalence of natural fasciolosis in cattle increased from 1990 to 1993 (13.6% to 25.2%) and diminished afterwards up to 1999 (at 12.6%). Those of natural paramphistomosis showed a progressive increase between 1990 and 1999 (from 5.2 to 44.7%). The prevalences of natural infections and the numbers of free rediae counted in the snails (*Lymnaea truncatula*) infected with *F. hepatica* did not show any significant variations over time. By contrast, the prevalences of natural paramphistomosis in snails significantly increased from 1996 to 2000 and remained after wards in the same range of values (3.7–5.3%), while the number of free rediae significantly increased up to 2006 (from a mean of 6.5 to 13.8 rediae per infected snail, respectively) (*Mage et al., 2006*).

A cross sectional study was carried out with the aim of determining the prevalence and intensity (worm burden) of *Paramphistomum* in ruminants slaughtered from October, 2010 to April, 2011 at Hashim Nur's Ethiopian Livestock and Meat Export industrialized abattoir in Debre Zeit, Ethiopia. One thousand one hundred fifty two ruminants comprising cattle, sheep and goats (n=384 each) were subjected to routine post mortem examination for the presence of *Paramphistomum*. The overall prevalence of *Paramphistomum* infection in the study proved to be 28.6 % (329/1152) of which 154 (40.1 %) were in cattle, 111 (28.9 %) in sheep and 64 (16.7 %) in goats. the highest prevalence of paramphistomosis was registered in highland goats, 30.2% (116/384) compared to those originated from lowland, 15.4 % (59/384). In the current study the prevalence proved to be higher in adult goats than young goats with prevalence of 30.5 % (117/384) in adult and 15.1% (58/384) in young goats. Infection was found to be highest in poor body condition (76.3 %), followed by medium (23.9 %) and good (6.9 %) body conditioned animals. A statistically significant difference ($p<0.05$) of Paramphistomosis prevalence was observed on the basis of species, body condition, different age groups and agro climatic zones (origins) of shoats. (*Melaku,et al.2012).*

A cross-sectional study was conducted to determine the prevalence and risk factors associated with small ruminant helminthiasis in north Gondar zone, northwest Ethiopia from November-January, 2008. A total of 558 small ruminants (458 sheep and 100 goats) were examined using standard parasitological procedures. The study revealed that the overall prevalence of helminthiasis was 47.67%. The species level prevalence of helminthiasis was 46.07% and 55% in sheep and goats, respectively. Sex and

age of the animals were found to have association with prevalence but significant differences were not found. Therefore during control and treatment of small ruminant helminthiasis agroecology, species, age and sex of the animals should be considered as potential risk factors for the occurrence of the disease in the study areas (*Dagnachew et al 2011)*

An epidemiological survey of paramphistomosis in ruminants in different districts of Punjab was conducted during the year 2005-2006 under DST, New Delhi sponsored project. A total of 1941 faecal samples (351 cattle, 791 buffaloes, 435 sheep and 364 goats) were collected from different village(s)/area(s) of the district of Punjab (Faridkot, Jalandhar, Ludhiana, Mansa, Muktsar, Nawanshahar and Sangroor). The samples were tested for paramphistome eggs by sedimentation method. Out of the total, 44 faecal samples (25 buffaloes, 7 cattle, 9 sheep and 3 goats) were found positive for paramphistome eggs with an incidence rate of 2.27%. The highest incidence was found in buffaloes (3.16%) followed by sheep (2.07%), cattle (1.99%) and goats (0.82%) in different district of Punjab. District-wise incidence rate was observed to be highest in Faridkot (7.4%) followed by Muktsar (2.37%), Mansa (2.3%), Sangroor (2.2%), Jalandhar (1.3%), Nawanshahar (1.3%), and Ludhiana (0.71%). Overall, seasonal epidemiology revealed highest incidence during monsoon with the incidence rate of 3.07% followed by 1.23% in winter, 0.6% in post-monsoon and 0.56% in summer (*Shabih1 et al.,2006*).

Another study was designed to compare information on parasitic diseases occurrence in nomadic cattle herds in Abyei area. faecal and blood samples collected from animals over one year . Faecal samples, blood smear, ticks and biting flies were collected over year. The results obtained

that the faecal samples from cattle examined by floatation and sedimentation methods showed that: Paramphistomum sp. constituted 11.25%, Fasciola gigantica 5.00%, Schistosoma bovis, 1.50%, Oesophagustomum sp. 2.50%, Moniezia sp. 0.63% and Eimeria sp. 4.38%. The occurrence of internal parasites was found higher during the wet season (*Gad Alkareem et al., 2012*).

A cross-sectional study was conducted to determine the prevalence and risk factors associated with The IgG antibody response to Calicophoron daubneyi (Digenea: Paramphistomidae) excretory/secretory antigens was evaluated in naturally infected cattle from Lugo (Galicia, NW Spain) by using an ELISA procedure. Five hundred .twenty four belong to the age group was surveyed G-1 (0–2 years old), G-2 (3–5 years old) and G-3 (>6 years old). The ELISA procedure showed that 61.2% of the cattle in the study had been exposed to the trematode, but only 10.1% passed eggs in the feces. (P. Dı´az,*et al*.2006).

To investigate the prevalence of amphistome parasites in Black Bengal goats slaughtered at different slaughterhouses of Mymensingh district, a total of 144 gastro-intestinal tracts were examined during the period of July 1998 to June 1999 in the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. Out of 144 Black Bengal goats, 105 (72.92%) were infected with a single or multiple species of amphistomes. Age had a significant ($p<0.01$) influence on the prevalence of amphistomes in goat. A higher prevalence (89.58%) was observed in olde animals followed by young ones (78.57%), whereas a lower prevalence (45.0%) was recorded in growing animals. However, the prevalence increased with the increase of age. Female animals (75.0%) were found more (1.44 times)

susceptible to amphistomes infection than males (67.5%). The prevalence of amphistomes was very high all the year round and the rate of infection was 83.64%, 69.23% and 64.0% during monsoon, winter and summer season respectively. It was concluded that Black Bengal goats are susceptible to amphistome infection irrespective of age, sex and season of the year. (*Uddin et al 2006).*

A Survey of prevalence and fluke burden of *Paramphistomum* sp. was conducted among the major ruminants slaughtered in Sokoto in Nigeria Central Abattoir between May and October, 2007. One hundred (100) of each were examined for the presence of *Paramphistomum* species (stomach flukes). Flukes were counted to determine the average fluke burden and prevalence. Out of the 300 animals, a total of 100 animals (33.3%) were infected with average fluke burden of 4794. Among which, 56 were cattle, with fluke burden of 2517(52.5%}, (32%) were sheep with fluke burden of 1907 (39.8%) and 12 goats with fluke burden of 370. and Out of 100 cattle, 20 (20%) males and 36 (36%) females were infected with flukes. Also, out of the 100 sheep, 4 (4%) were males and 28 (28%} were females and in goats, 4 (4%) were males and 8 (8%) were females. On the basis of age the results showed that 4 cattle(7.1%) out of the 56 infected animals were 1-2 years, 40 (71.4%) were 3-4 years old and 12 (21.4%) were >4 years . Of the 32 infected sheep, 6 (18.7%) were 1-2 years, 18(56.2%) were 3-4 years and 8(25%) were >4yrs. Similarly, 2(16%) out of the 12 goats infected were 1-2 years, 8 (66.6%) were 3-4 years old and 2 (16%) were >4 years. The result obtained showed that *Paramphistomiasis* is prevalent in the

cattle in the area, with female cattle having higher prevalence.*(ABunza et al 2008)*

In another study investigating the role of snail in lifecycle of *paramphitoma* single-miracidium infections of *Lymnaea truncatula* with *Paramphistomum daubneyi* or with *Fasciola hepatica* were carried out under laboratory conditions to count free rediae, their germinal embryos, and to determine the cercarial productivity of each redial generation. In snails infected by *P. daubneyi*, the cercariae were produced by the first (8.7 cercariae per redia) and second (8.9 per redia) generations. At day 63 post-exposure, they corresponded, respectively, to 53.9% and 46.1% of cercariae produced by all rediae. In snails infected by *F. hepatica*, the majority of cercariae were produced by the R2a group (18.2 cercariae per redia) and corresponded to 66.0% of cercariae produced all rediae.The cercariae produced by the other redial groups were more limited in number: 17.5 per redia in the R1b group (28.7%) and 2.0 per redia in the R2b/R3a group (5.3%). Cercarial productivity of *P. daubneyi* until day 63 post-exposure was more limited in number than that of *F. hepatica*: a total of 145 cercariae per snail versus 427 per snail.(*Abrous et al., 2000*).

in another study of italian Isolates of the rumen fluke Calicophoron daubneyi (Digenea: Paramphistomidae) from various hosts in three locations in southern Italy were characterized genetically. The second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) plus flanking 5.8S and 28S sequence (ITS-2+) was amplified from individual rumen flukes by PCR. PCR-linked restriction fragment length polymorphism (PCR-RFLP) analysis was performed using four different restriction endonucleases, and PCR products were sequenced. The PCR analyses from all the C. daubneyi

specimens produced identical fragments, and the PCR-RFLP analyses did not show, among of the four restriction endonucleases, the differences between the C. daubneyi specimens. The sequence analyses of the ITS-2+ fromeach of the C. daubneyi specimens showed that all of them 428 bp, and composed of the entire ITS-2 sequence (282 bp) plus the two partial flanking conserved sequences, 5.8S (99 bp) and 28S (47 bp). No intra-specific variation was observed in the nucleotide composition of the ITS-2+ (homology = 100%). There was, however, an observable interspecific variation between the ITS-2+ of C. daubneyi and the ITS-2+ of both Calicophoron calicophorum(homology = 97.2 %) and Calicophoron microbothrioides (homology = 97.4 %), both previously deposited in the GenBankTM. The findings of the obtained study showed that, ITS-2 can serve as an effective genetic marker for the molecular identification of paramphistomes, and as a useful tool for developing molecular epidemiological techniques for the study of C.daubneyi transmission patterns and prevalence in definitive and intermediate hosts (*Rinaldi et al., 2005*).

Ageographic information system (GIS)was constructed using remote sensing (RS) and landscape feature data together with *Calicophoron daubneyi* positive survey records from 197 georeferenced ovine farms with animals pasturing in a 3971 km2 area of the southern Italian Apennines. The objective was to study the spatial distribution of this rumen fluke, identify environmental features that influence its distribution, and develop a preliminary risk assessment model. The GIS for the study area was constructed utilizing the following environmental variables: normalized difference vegetation index (NDVI), land cover, elevation, slope, aspect, and

total length of rivers. These variables were then calculated for “buffer zones” consisting of the areas included in a circle of 3 km diameter centered on 197 farms.The environmental data obtained from GIS and RS and from data taken by the veterinarians on the field (stocking rate and presence of streams, springs and brooks on pasture) were analyzed by univariate (Spearman and ANOVA) and multivariate (discriminant) statistical analyses using the farm coprological status (positive/negative) as the dependent variable. Sheep on 32 of the 197 (16.2%) farms, were positive for *C. daubneyi*, with an average intensity of 52 epg (*Cringoli et al., 2004*).

Another investigation was carried out From November 1998 to October 2000, in the patterns of distribution and seasonal population fluctuations of snails and factors influencing them were investigated in six dams and six streams in the highveld region, in nine dams in the lowveld region of Zimbabwe. At monthly intervalsIn total, 13 gastropod specimens representing eight genera and 10 species were collected during the study period, with pulmonates contributing seven species . The number of pulmonates was 16.4 times the number of prosobranch snails. *Bulinus tropicus* contributed 31.4%, *Lymnaea natalensis* 25.5%, *B.globosus* 22.6% and *Biomphalaria pfeifferi* 19.5% of pulmonate snails. *Bulinus forskalii*, *Gyraulus costulatus* and *Ceratophallus natalensis* together contributed 1% of the pulmonates (*Chingwena at al., 2000*).

In an attempt to establish an ideal method for mass production of *Calicophoron microbothrium* metacercariae, a study was carried out to compare the shedding capacities of *Bulinus tropicus* naturally and experimentally infected with *C. microbothrium.* A total of 906 F1 *B. tropicus* between 4 and 5 weeks old were each experimentally infected with

two *C. microbothrium* miracidia and monitored for 12 weeks. The infected snails were fed on dried lettuce and fish flakes and were kept in 1 l plastic aquaria housed in a snail room where temperature, light and humidity were controlled. Seventy-four percent of the experimentally infected snails died during the prepatent period and of the remaining, only 13.2 % developed patent infection, while 12.5 % were refractory. Snail growth rate was poor and the average shedding rate was 20 cercariae per snail per day (*Mavenyengwai et al., 2006*).

Rumen of 100 slaughtered animals viz. sheep (n=14), goats (n=42), cattle (n=34) and buffalo (n=10) were examined to determine the prevalence of adult *Paramphistomum cervi* during January 2007 in Tehsil Jatoi, District Muzaffar Garh, Pakistan. Overall prevalence was found to be 22% (22/100) and species wise prevalence was 28.57% (4/14) in sheep, 23.80% (10/42) in goats, 17.64% (6/34) in cattle and 20% (2/10) in buffaloes, the difference between the species was not significant. (*Raza et al 2009*).

To investigate the Epidemiology of *Paramphistomum* infection in cattle, faecal samples from 360 cattle were collected from individual areas of the Sirajgonj district from March 2009 to April 2010. One hundred and ninety one animals (53.1%) were infected with single or multiple species of *Paramphistomum*. Age of animals significantly ($P<0.05$) influenced the prevalence of Paramphistomiasis. Older animals suffered (60.3%) more than growing (44.4%) and young (54.0%) ones. Older animals were 1.94 times more susceptible than growing animals. Furthermore, females were more (59.5%; 1.79 times) susceptible to

Paramphistamum spp. than males (45%). Breed has also significant ($p<0.05$) effect. The prevalence of Paramphistomiasis was higher ($p<0.05$) in crossbred (61.8%) animals than that of local (49.2%) cattle*. (paul etal 2011).*

The analysis of infection by Paramphistomidae trematodes was conducted in two agricultural regions with different knowledge on this parasitosis. Faecal and blood samples were collected from 374 cattle in Salto (NW Uruguay) where there is a lack of information about paramphistomosis. A total of 429 cattle from Galicia (NW Spain), he percentage of cattle passing Paramphistomidae-eggs by faeces was 7% (95% Confidence Interval 5, 10). A significantly higher prevalence of paramphistomosis in the Hereford Angus cattle (OR = 3.5) was recorded (*Sanchís etal2013) .*

A cross sectional study was carried out from October 2010 to March 2011 at Andassa Livestock Research Center, North-West Ethiopia. The objective was to determine the prevalence of cattle flukes infection. Faecal samples were collected from a total of 384 cattle, cross breed (n= 39) and Fogera breed (n=345) of all age groups and sex. Sedimentation technique was employed for the recovery of fluke eggs from freshly collected fecal sample. The results indicated that the overall prevalence of bovine flukes infection was 60.42%. In this study, the highest prevalence was recorded from Paramphistomosis (45.83%) followed by

Fasciolosis (23.96%), and Schistosomosis (9.89%). (*Yenenehet al 2012).*

A total of 2628 faecal samples (651 cattle, 1608 buffaloes, 213 sheep and 156 goats) were collected randomly from different village(s)/area(s) of the district of Punjab and adjoining areas in Jammu (J&K) during the period July 2004 to June 2005. The samples were screened microscopically for paramphistome eggs by sedimentation methods. Out of the total, 167 fecal samples (122 buffaloes, 22 cattle, 14 sheep and 9 goats) were found positive for paramphistome eggs with an incidence rate of 6.35 percent. The highest incidence was found in buffaloes followed by sheep, goats and cattle. District-wise incidence rate was observed to be highest in Gurdaspur followed by Amritsar, Kapurthala and Jammu (*shabeh et al 2006).*

Epidemiological studies were undertaken at slaughter houses, livestock farms, veterinary hospitals and on house hold buffaloes under different management and climatic conditions in four different districts of the Punjab province. Infection rate was 7.83%, 12.33%, 7.17% and 4.25% respectively in the cattle at the slaughter house, livestock farm, veterinary hospital and at household cattle. Overall the highest prevalence in terms of season, 26% and 14.50%, was recorded during autumn at livestock farms and slaughtered cattle followed by 9.75% veterinary hospitals

during summer and the lowest (2.5%) in household cattle was recorded during winter. *(khan et al 2008).*

A Survey of prevalence and fluke burden of Paramphistomum sp. was conducted among the major ruminants slaughtered in Sokoto Central Abattoir between May and October, 2007. One hundred (100) of goats, sheep and cattle each were examined for the presence of Paramphistomum species (stomach flukes). Flukes were counted to determine the average fluke burden and prevalence. Out of the 300 animals, a total of 100 (33.3%) were infected with an average fluke burden of 4794. Out of these, 56 (56%) were cattle, with fluke burden of 2517(52.5%}, 32 (32%) were sheep with fluke burden of 1907 (39.8%) and 12 (12%} with fluke burden of 370 (6.7%) were goats. Out of the 100 cattle, 20 (20%) males and 36 (36%) females were infected with flukes. Also, out of the 100 sheep, 4 (4%) were males and 28 (28%} were females and in goats, 4 (4%) were males while 8 (8%) were females. On the basis of age the result showed that 4(7.1%) out of the 56 infected animals were those of 1-2 yrs, 40 (71.4%} were 3-4 yrs old and 12 (21.4%) were animals >4 yrs in respect of cattle. Of the 32 infected sheep, 6 (18.7%) were 1-2 yrs, 18(56.2%) were 3-4 yrs and 8(25%) were >4yrs. Similarly, 2(16%) out of the 12 goats infected were 1-2 yrs, 8 (66.6%) were 3-4 yrs old and 2 (16%) were >4 yrs (*abunza et al.,2008*).

The present study explored various basic aspects of the epidemiology of paramphistomosis in Galicia, the main cattle producing region in Spain. A total of 589 cows from different farms located across the region were selected at random in the slaughterhouse for examination of the rumens and reticula for the presence of Paramphistomidae flukes. Paramphistomes were found in 111 of 589 necropsied cows

(18.8%; 95% CI: 15.7%-21.9%), with higher prevalences of infection in beef cows than in dairy cows (29.2% vs 13.9%). Although the number of flukes per animal was generally low (median= 266 flukes), some cows harboured large parasite burdens (up to 11895 flukes), which may have harmful effects on their health or productivity (*Gonz´alez et al 2012)*

Chapter Two

Materials and Methods

2.1 Study Area:

Study was conducted on Rabak slaughter house, (The capital of White Nile State of Sudan) White Nile State has strategic location, it lies in the South of North Sudan, (Latitude: 13° 16' 27" N and Longitude: 32° 26' 59" E),

population of the state is estimated at 1.73 millions of inhabitants (*NBHS, 2009*), about 2/3 of them live in rural area , The potential of White Nile area for grazing is varied from one area to another, and mostly dependant on the availability of vegetation and water located on the Eastern bank of the White Nile river (*Musa et al 2013*) . The estimated livestock in White Nile state is approximately 8 million heads, which are concentrated in the water accessible areas of western and eastern parts of the state. Cattle constitute the significant animal wealth in further southern partsAgro-ecologically, the State is within the semi-desert zone, characterized by sandy areas in various location and with annual rainfall varying from 300 mm in the north to 600 mm in the south (*FAO/WFP, 2009*).

Rabak is one of major commercial cities in the Sudan it lies between latitude 13°1048 north to longitudinal 32°44.25 east. it is an Agricultural irrigated area. Specially on sugar cane and a center for cotton trade . Rabak is located on the eastern bank of the White Nile river, approximately 260 km south of Khartoum and 340 km west of Ethiopia. Rabak lies some 362 meters above sea level.

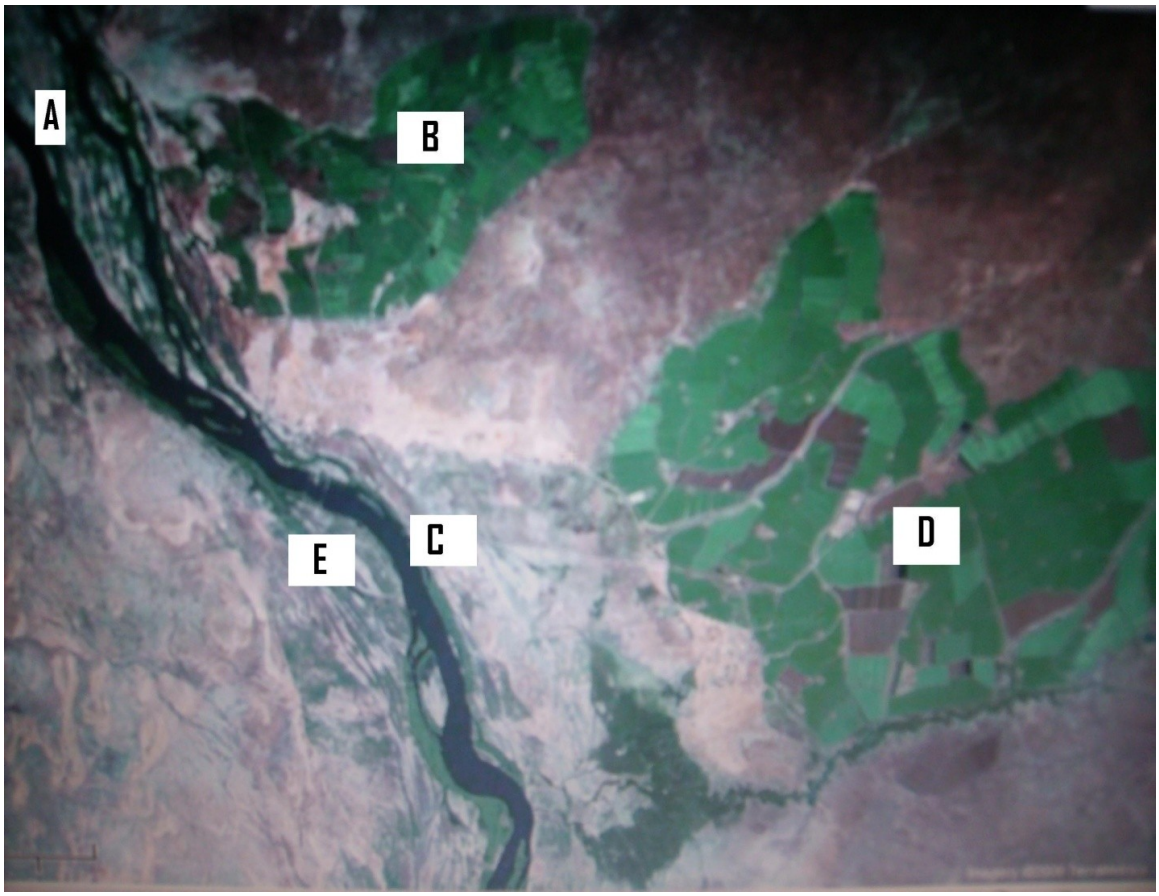

Figure:(5) Satallite picture for the study area, A: Gezira Abba, B:Asalaia Suger company, C: Rabak Town, D: Kenana Suger company, E: Kosti Town. (*mukhtar et al 2010*)

The Climatic condition in rabak like most of Sudan, has a very dry climate. The climate stays hot throughout the year, even into November temperatures still break high degrees.

2.2 The study design:

The study design was a cross sectional study which provides snapshot information on occurance of a disease (Martin et al. , 1987). A Cross-sectional study was conducted at Rabak abattoir on three randomly

selected days .These days was selected were Sunday,Tuesday and Thursday. The animals in these days selected by systematic random sampling method. Among each group of five animals were selected one animal was examined

2.3 Sample Size *:*

The expected prevalence of cattle paramphistomiasis for calculation of sample size was taken from the study in sudan (Study on prevalence of parasitic diseases in cattle in Abyei area – Sudan) in which the prevalence of paramphistomiasis in cattle was 11.25% (Gad alkareem et 2012).

Sample size was calculated according to the formula by *Martin et al.1988*

$$n = \frac{4\,P^{X}Q}{L^{2}}$$

Where:

n≡ Required Sample Size P≡ Expected prevalence = 0.04

Q ≡ 1- P = 0.96 L≡ Allowable error

4*11*89*10.000 =156 animal

100*100*25

2.4. Individual risk factors:

Potential individual risk factors and their categories were as follow:

sex (male, female) , age (adult , young), breed (local and cross), body
condition (good ,poor),

2.5 Management risk factors:

Management risk factors include: grazing type(indoor, outdoor), source of animals(kordofan ,white Nile) , water source(tap , canal ,river), snail presence (yes , no), water bodies (yes , no), vegetation (yes , no), knowledge of owner about disease (yes , no), manure disposal (yes , no), fasciola (positive , negative), schitiosoma (positive , negative),and other disease (positive , negative).

2.6 Animals and sample collection:

Survey of paramphistomiasis in slaughter house:

Samples were collected in the abattoir of Rabak (White Nile state, Sudan) Rectal faecal sample and 9 ml of blood was collected from the jugular vein in vaccutainer tube. The date, the number of total infected animals and the age also recorded.

The majority of the slaughtered cattle were beef cattle and minorities were culled dairy cows slaughtered . After collection, samples were transported to the laboratory. The feces were stored at 4 ^{0}C until the test was performed within 48 h. The sera were separated from the blood samples and stored at -20 ^{0}C until use

2.7 Diagnostic techniques:

2.7.1 Fecal Examination:

fecal samples (approximately 10 gram) were collected directly from the rectum of the animal in a clean plastic container After labeling with specific identification number, each sample was transported to Rabak veterinary research laboratory. Fecal samples were examined by sedimentation technique for the presence of fluke eggs using the method described by *Adejoju et al*., (2008). the technique was performed on 10 g of feces to which. 200 ml water was added and mixed. The mixture was filtered 3 times through a specific sieve. The filtrate was allowed to stand for 10 min after which the sediment was collected in a test tube and centrifuged at 700 rpm for 3 min. After centrifugation, the supernatant was decanted and a drop of the sediment was tested microscopically. Trematode eggs were identified on the basis of morphology (*Soulsby, 1982*).

Figure 6: Eggs of Paramphistomum (P) cervi and Fasciola hepatica (F) *(wall2012)*

2.7.2 Serological examinations:

2.7.2.1 Collection and preparation of sera:

The vaccutainer tubes in which blood was collected were kept in an upright position at room temperature for about 2 hours. The serum was separated, kept in screw capped plastic vials and transported to the Veterinary Research Laboratory in Rabak . The sera were stored at -20ºC till further use, which on inspection were found positive, were labelled "paramphistoma positive" and those from animals without were labelled "paramphistoma negative".

2.7.2.2 Excretory and Secretory (E/S) Antigen:

Adult paramphistoma were collected from livers of infected cattle, and washed until they were free of any visible bile pigments. This was achieved by first washing in six changes in warm(37 ^{0}C) sterile phosphate buffered saline (PBS), followed by six changes in sterile PBS containing penicillin (100 IU/ml), streptomycin (100 mg/ml) and fungizone (2 mg/ml) and finally six changes in medium containing penicillin (100 IU/ml), streptomycin (100 mg/ml) and fungizone (2 mg/ml). The washed live flukes were placed into tissue culture flasks containing medium with penicillin (100 IU/ml), streptomycin (100 mg/ml), fungizone (2 mg/ml) at one fluke per 5 ml of medium. The flasks containing the flukes were incubated at 37 ^{0}C for 24 h.After which, the medium was collected and centrifuged at 2000 g, for 30 min. The supernatant was collected and the protein concentration content was determined. The protein concentration of the E/S products was estimated by the method described by *Lowery, et al., (1951*). The solution containing excretory and secretory products, then aliquoted into 1 ml volumes and stored at -20 ^{0}C until used.

2.7.2.3Enzyme linked immunosorbant assay (ELISA)

Procedure of Indirect ELISA:

The method used as described by Estuningsih *et al*., 2004 with some modification, The antigen was diluted in coating buffer at optimal

concentration. Optimal concentration was determined after preparation of different antigen concentration and different dilutions of sera and conjugate (chequer-board titrations). The optimal concentration was 5 µg/ml coating buffer for the E/S antigen, and 1:200 the dilution of sera, 1:20 000 for the conjugate.Each well of a polystyrene microtiter plates (Maxisorp, Nunc) was filled with 100 µl of the antigen and the plates were incubated overnight at 4 ^{0}C. The plates were washed 3 times with PBST to get rid of excess unbound antigen and the remaining free binding sites are blocked with blocking buffer; 200 µl/well for 1 hour.The plates were then washed 3 times with PBST. Tested sera were added to the plates (100 µl/well, 1: 200 dilution in blocking buffer,at pH 7.4) and incubated at 37^{o}C for 1 hour .The plates were then washed 3 times with PBST and 100 µl/well of the conjugate (1:20 000 dilution in blocking buffer, at pH 7.4) were added to all wells and incubated for 45 min at 37^{o}C.After incubation the plates were washed 5 times with phosphate buffered saline (PBS), 100 µl of the substrate were added to all wells. The plates were incubated for 10 minutes at 37^{o}C.The reaction was stopped by adding 50 µl/well of 2 M sulphuric acid. The enzyme mediated color reaction was measured at 450 nm using a Multiskan ELISA reader.

2.8 Analysis of the results:

Results of the study were analyzed using statistical package of social science (SPSS). First, Descriptive statistical analysis was displayed in frequency distribution and cross tabulation tables . Univariate analysis using the chi-square for qualitative data . P-value of 0.3 was considered as significant association and the risk factor was then selected to enter the multivariate analysis . Multivariate analysis : Forward or backward stepwise logistic regression was used to analyse the data and to investigate

association between a potential risk factor and the prevalence of hydatidosis . A p-value of 0.05 indicated significant association between paramphistomiasis and the risk factor.

Chapter Three

Results

3. Descriptive statistical analysis frequency , cross tabulation and association between paramphistomiasis diagnosed by sedimentation test and potential risk factor:

3.1 Results:

Of the total 156 cattle inspected, 46 (29.5%) animals were positive, and the rest were negative for paramphistomiasis (table 3.1.1). the overall prevalence of cattle paramphistomiasis examined by fecal sedimentation test in Rabak was 29.5%.

Table 3.1.1: Distribution of paramphistomiasis infection among 156 cattle examined by fecal sedimentation test in Rabak slaughterhouse

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
-ve	110	70.5	70.5	70.5
+ve	46	29.5	29.5	100.0
Total	**156**	**100.0**	**100.0**	

3.2 Sex of animal:

The results of this study showed that the distribution of 156 cattle examined for paramphiistoiasis according to sex. Total number of male examined was 34 (21.8%) animals, while the total number of female examined was 122 (78.2%) (table 3.1.2). Among males, 9 animals were found infected. Rate of infection within males was 26.5%. While among females, 37 animal were found infected. The rate of infection within females was 30.3% (table 3.1.3).

The Chi- square test showed no significant association between paramphistomum infection and sex of animal (p-value = 0.663),(table 3.1.4).

3.3 Age of animal:

One hundred fifty six cattle of various ages were examined in this study. The result showed that the age distribution of cattle, 83 (53.2%) were less than or equal 5 years (young) and 73 (46.8%) of cattle were more than 5 years (old), (table 3.1.2). Among young animals 27 animals were found infected. The rate of infection within young animals was 32.5%. However

among adults 19 animals was found infected. The rate of infection within adults was 26% (table 3.1.3).

The Chi- square test showed no significant association between paramphiistoimum infection and age of animal (p-value = 0.37),(table 3.1.4).

3.4 Breed:

The results of study showed distribution of paramphistomiasis infection in rabak slaughter house according to breeds. Total number of local breed was 88 (56.4%) animal. Among these 88 animals, 20 animals were found infected. The rate of infection was 22.7%. Total number of cross breed examined was 68 (43.6%). Among these, there was 26 infections. The rate of infection was 38.2%

The Chi- square test showed no significant association between the infection and breed (p-value = 0.788), (table 3.1.4).

3.5 Body condition:

The body condition of animals and the presence of infection were investigated. Eighty three (53.2%) of cattle were found to be in good condition, while 73(46.8%) of cattle were found to be in poor condition (table 3.1.2). Among good condition animals 17 was found infected. The rate of infection within good animals was 20.4%. However 29 animals was found infected among poor condition animals. The rate of infection within poor animals was 39.7% (table 3.1.3).

The Chi- square test showed a significant association between the infection and body condition (p-value = 0.009),(table 3.1.4).

3.6 Grazing:

The results of study showed that the distribution of paramphistoiasis infection in Rabak slaughter house according to grazing. Total number of indoor grazing was 61 (39.1%) animals. Among these 61 animals, 25 were found infected. The rate of infection was 40.9%. Total number of outdoor grazing examined was 95 (60.9%). Among these, there was 21 infection. The rate of infection was 22.1% (table 3.1.3).

The Chi- square test showed a significant association between the infection and grazing (p-value = 0.012), (table 3.1.4)

3.7 Source of animal:

Of the total 156 cattle inspected, only one animal was from Kordofan which was not infected, One hundred fifty five (99.4%) were from the White Nile. Among these 155 animals, 46 were found infected .The rate of infection in Kordofan was 1 (.6%). And the rate of infection in White Nile was (29.7%),(table3.1.3).

The Chi-square results showed that there is no significant association between the infection and source of animal (p-value = 0.51), (table 3.1.4).

3.8 Water source:

The results of study showed that the distribution of paramphistoiasis infection in Rabak slaughter house according to water source. Total number of animals drinking from taps was 65 (41.7%) animals. Among these 65 animals, 22 were found infected. The rate of infection was 34%. Total number of animal drinking from canal examined was 56 (35.9%). Among

these 56 animals there was 10 infections. The rate of infection was 17.9%. (table 3.1.3). Total number of animals drinking from river was 35 (22.4%) animals. Among these 35 animals 14 were found infected. The rate of infection was 40%(table 3.1.3).

The Chi- square test showed no significant association between the infection and water source (p-value 0.516), (table 3.1.4).

3.9 Presence of snails:

The results of study showed that the distribution of paramphistomasis infection in Rabak slaughter house according to presence of snails. total number of animals with no presence of snails examined were 37 (23.7%) animals. while the total number of animals with presence of snails examined were 119 (76.3%) (table3.1.3).Among these with no presence of snails 3 animals found infected .The rate of infection with no presence of snails was (8.1%) . Among these with presence of snails 43 animals found infected .The rate of infection with presence of snails was (36.1%) .

The Chi- square test showed significant association between the infection and presence of snails (p-value = 0.001), (table 3.1.4).

3.10 Presence of water bodies:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughter house according to presence of water bodies .Total number of animals with no presence of water bodies examined were 44 (28.2%) animals. while the total number of animals with presence of water bodies examined were 111 (71.2%) animals(table 3.1.3).Among these with no presence of water bodies 9 animals found infected .The rate of

infection with no presence of water bodies was (20.4%). Among these with presence of water bodies 37 animals found infected .The rate of infection with presence of water bodies was (33.3%) .

The Chi- square test showed no significant association between the infection and presence of water bodies (p-value = 0.23), (table 3.1.4).

3.11: Vegetation:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughterhouse according to vegetation. Total number of animals

with no vegetation were 27 (17.3%) animals. while the Total number of

Animals with vegetation examined were 129 (82.7%) animals (table3.1.3).Among these with no vegetation 7 animals found infected .The rate of infection with no vegetation was (25.9%) . Among these with vegetation 39 animals found infected .The rate of infection with vegetation was (30.2%) .

The Chi- square test showed no significant association between the infection and vegetation (p-value = 0.65), (table 3.1.4).

3.12 Knowledge about disease:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughter house according to knowledge about disease. Total number of animals with no knowledge of owner about disease

examined were 34 (21.8%) animals. while the total number of Animals with knowledge of owner about disease examined were 122 (78.2%) animals (table3.1.3).Among these no knowledge of owners about disease 7 animals found infected .the rate of infection with no knowledge of owner about disease was (20.9%) . Among these with knowledge of owner about disease 39 animals found infected .The rate of knowledge of owner about disease infection with knowledge of owners about disease was (31.9%) .

The Chi- square test showed no significant association between the infection and knowledge about disease (p-value = 0.19), (table 3.1.4).

3.13 Manure disposal:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughter house according to manure disposal. total number of animals With no manure disposal examined were 133 (85.3%) animals, while the total number of animals with manure disposal examined were 23 (14.7%) animals (table3.1.3).Among these with no manure disposal 43 animals found infected .The rate of infection with no manure disposal was (32.3%) . Among these manure disposal 3 animals found infected .the rate of infection with manure disposal was (13%).

The Chi- square test showed no significant association between the infection and manure disposal. (p-value = 0.06), (table 3.1.4).

3.14 *Fasciola*:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughter house according to infection of animals with fascioliasis. total number of animals with negative fasciolaisis examined

were 125 (80.1%) animals. while the total number of animals with positive fasciolasis examined were 31(19.9%) animals (table3.1.3).Among these negative fasciolasis 37 animals found infected .the rate of infection with negative fasciolasis was (26.9%) . Among these positive fasciolasis 9 animals found infected .the rate of infection with positive fascioliasis was (29%).

The Chi- square test showed no significant association between the infection and fascioliasis (p-value = 0.95), (table 3.1.4).

3.15 *Schistosoma*:

The results of study showed that the distribution of paramphistomiasis infection in Rabak slaughter house according to infection of animals with schistosomiasis. Total number of animals with negative schistosomiasis examined were 154 (98.7%) animals. while the Total number of animals with positive schitosomiasis examined were 2 (1.3%) animals (table3.1.3).Among these negative schistosomiasis 46 animals found infected .The rate of infection with negative schistosomiasis was (30%) . Among these positive schitosomiasis 2 animals found infected .the rate of infection with positive schitosomiasis was (100%).

The Chi- square test showed no significant association between the infection and schitosomiasis (p-value = 0.35), (table 3.1.4).

3.14 Other diseases:

The results of study showed distribution of paramphistomiasis infection in Rabak slaughter house according to infection of animals with other diseases. Total number of animals with negative other diseases examined were146

(93.6%) animals. while the total number of Animals with positive to other diseases examined were10 (6.4%) animals (table3.1.3).Among these negative to other diseases examined 46 animals found infected .the rate of infection in negative to other diseases examined was(31.5%) . Among these positive to other diseases no animals were found infected .The rate of infection with positive to other diseases was (0%).

The Chi- square test showed significant association between the infection and other diseases (p-value = 0.03), (table 3.1.4)

Table 3.1.2: Summary of frequency distribution of 156 cattle from Rabak slaughterhouse examined for paramphistomiasis by fecal sedimentation test according to potential risk factors

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Sex			
Femal	122	78.2	78.2
e	34	21.8	100.0
Male			
Age (years)			
Young(≤5)	83	53.2	53.2
Old(>5)	73	46.8	100.0
Breed	88	56.4	56.4
local	68	43.6	100.0
cross			
Body condition			
Poor	73	46.8	46.8
Good	83	53.2	100.0
Grazing type			
indoor	61	39.1	39.1
outdoor	95	60.9	100.0

Source			
Kordofan	1	.6	.6
White Nile	155	99.4	100.0

Table 3.1.2 continued :

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Water source			
Tap	65	41.7	41.7
Canal	56	35.9	77.6
Rever	35	22.4	100.0
Snails presence			
No	37	23.7	23.7
Yes	119	76.3	100.0
Water bodies			
No	45	28.8	28.2
Yes	111	71.2	71.2
Vegetation			
No	27	17.3	17.3
Yes	129	82.7	100.0
Knowledge			
No	34	21.8	21.8
Yes	122	78.2	100.0

Manure disposal			
No	133	85.3	85.3
Yes	23	14.7	100.0
Fasciolaiasis			
-ve	125	80.1	80.1
+ve	31	19.9	100.0

Table 3.1.2 continued :

Risk Factors	Frequency	Relative Frequency %	Cumulative Frequency %
Schitosomaiasis			
-ve	154	98.7	98.7
+ve	2	1.3	100.0
Other diseases			
-ve	146	93.6	93.6
+ve	10	6.4	100.0

Table 3.1.3: Summary of cross tabulation for the rate of paramphistomiasis in each category of the potential risk factors in 156 cattle from Rabak slaughterhouse examined by fecal sedimentstion test

Risk factors	No. inspected	No. affected (%)
Sex		
Female	122	37(30.3)
Male	34	9(26.5)
Age (years)		
Young(≤5)	83	27(32.5)
Old(>5)	73	19(26)
Breed		
local	88	20(22.7)
cross	68	26(38.2)
Body condition		
Poor	73	29(39.7)
Good	83	17(20.4)
Grazing type		
indoor	61	25(40.9)
outdoor	95	21(22.1)
Source		
Kordofan	1	0(0)
White Nile	155	46(29.7)
Water source		
Tap	65	22(34)
Canal	56	10(17.9)
Rever	35	14(40)

Table 3.1.3 continued:

Risk factors	No. inspected	No. affected (%)
Snails presence		
No	37	3(8.1)
Yes	119	43(36.1)
Water bodies		
No	44	9(20.4)
Yes	111	37(33.3)
Vegetation		
No	27	7(25.9)
Yes	129	39(30.2)
Knowledge		
No	34	7(20.6)
Yes	122	39(32)
Manure disposal		
No	133	43(32.3)
Yes	23	3(13)
Fasciola		
-ve	125	37(29.6)
+ve	31	9(29)
Schitosoma		
-ve	154	46(30)
+ve	2	2(100)

Table 3.1.3 continued:

Risk factors	No .inspected	No .affected (%)
Other diseases		
-ve	146	46(31.5)
+ve	10	0(0)

Table 3.1.4: Summary univariate analysis for The association between paramphistomiasis and potential risk factors in 156 cattle examined at Rabak slaughterhouse by fecal sedimentation test using the Chi_square test:

Risk factors	No. inspected	No. affected (%)	d.f	X^2 value	p- value
Sex			1	.190	.663
Female	122	37(30.3)			
Male	34	9(26.4)			
Age(years)			1	.790	.374
Young(≤5)	83	27(32.5)			
Old(>5)	73	19(26)			
Breed			1	4.437	.035
local	88	20(22.7)			
Cross	68	26(38.2)			
Body condition			1	6.918	.009
Poor	73	29(39.7)			
Good	83	17(20.4)			
Grazing type			1	6.367	.012
indoor	61	25(40.9)			
outdoor	95	21(22.1)			
Source			1	.421	.516
Kordofan	1	0(0)			
White Nile	155	46(29.6)			
Water source			2	6.097	.516
Tap	65	22(33.8)			
Canal	56	10(17.8)			
Rever	35	14(40)			

Table 3.1.4 continued:

Risk factors	No. inspected	No. affected (%)	d.f	Chi-square value	p- value

Snails presence No Yes	37 119	3(8.1) 43(36.1)	1	10.6	.001
Water bodies No Yes	44 111	9(20.4) 37(33.3)	1	2.934	.231
Vegetation No Yes	27 129	7(25.9) 39(30.2)	1	.199	.655
Knowledge No Yes	34 122	7(20.6) 39(31.9)	1	1.656	.198
Manure disposal No Yes	133 23	43(32.3) 3(13.04)	1	3.508	.061
Fasciola -ve +ve	125 31	37(29.6) 9(29.03)	1	.004	.951
Schitosoma -ve +ve	125 2	46(36.8) 2(100)	1	.847	.357

Table 3.1.4 continued:

Risk factors	No. inspected	No. affected (%)	d.f	Chi-square value	p- value

Other diseases -ve +ve	146 10	46(31.5) 0(0)	1	4.468	.035

Table 3.1.5: multivariate analysis for The association between paramphistomiasis and potential risk factors in 156 cattle examined at Rabak slaughterhouse by fecal sedimentation test

Risk factors	No. inspected	No. affected (%)	Exp(B)	p-value	95.0%C.I

					Low	High
Breed						
local	88	20(27.7)	Ref			
cross	68	26(38.2)	.565	.035	.224	1.488
Body condition						
Poor	73	29(39.7)	1.540		.660	3.594
Good	83	17(20.4)	Ref	.009		
Grazing type						
indoor	61	25(41)	2.071		.482	8.895
outdoor	95	21(22.1)	Ref	.012		
Water source						
Tap	65	22(33.8)	.825	.51	.158	4.29
Canal	56	10(17.8)	Ref	.313		
River	35	14(40)	.336		.101	1.11
Snails presence						
No	37	3(8.1)	Ref			
Yes	119	43(36.1)	.092	.001	.15	.551

Table 3.1.5 continued:

Risk factors	No.inspected	No.affected (%)	Exp(B)	P-value	95.0%C.I	
					Low	High
Knowledge						
No	34	7(20.5)	Ref			
Yes	122	39(31.9)	1.127	0.198	.380	3.343
Manure disposal						
No				0.061		

Yes 23	133 23	43(32.3) 3(13)	1.256 Ref		0.281	5.624
Other diseases -ve +ve	 146 10	 46(31.5) 1(10)	 Ref 2.17	.03		

3.2 Descriptive statistical analysis frequency tables, cross tabulation and association tables between the paramphistomiasis (diagnosed by ELISA) and risk factors:

3.2.1 Results:

Of the total 156 cattle inspected, 83 (53.2%) animals were positive, and the rest were negative for paramphistomiasis (table .3.2.1). the overall prevalence of cattle paramphistomasis examined by ELISA in Rabak was 53.2%

Table 3.2.1 Distribution of paramphistomiasis infection among 156 cattle examined by ELISA test in Rabak slaughterhouse:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
-ve	73	46.8	46.8	46.8
+ve	83	53.2	53.2	100.0
Total	**156**	**100.0**	**100.0**	

3.2.2 Sex of animals:

Among 122 females, 69 animals were found infected. Rate of infection within females was 56.5%, While among 34 males, 14 animals were found infected. The rate of infection within males was 41.1% (table 3.2.2).

The Chi- square test showed no significant association between paramphiistoimum infection and age of animal (p-value = 0.112),(table 3.2.3).

3.2.3. Age of animals:

. Among 83 young animals 42 animals were found infected. The Rate of infection within young animals was 50.6%. However Among 73 adult animals 41animals were found infected. Rate of infection within adults was 56.1% (table 3.2.2).

The Chi- square test showed no significant association between paramphiistoimum infection and age of animal (p-value = 0.487),(table 3.2.3).

3.2.4. Breed:

Among 88 local breed animals, 45animals were found infected. The rate of infection was (51.8%). Among 68 cross animals, 38 were infection. The rate of infection was (55.8%) (table 3.2.2)

The Chi- square test showed no significant association between the infection and breed (p-value = 0.556), (table 3.2.3).

3.2.5. Body condition:

Among 83 good body condition animals 42 were found infected. The rate of infection within good animals was (50.6%). However 41 animals were found infected among 73 poor body condition animals. The rate of infection within poor animals was (56.1%) (table 3.2.2).

The Chi- square test showed no significant association between the infection and breed (p-value = 0.487), (table 3.2.3).

3.2.6. Grazing:

Among 61 indoor grazing animals, 35 were found infected. The rate of infection was (73.3%). While among 95 outdoor grazing animal there was 48 were infection. The rate of infection was (50.5%) (table 3.2.2).

The Chi- square test showed significant association between the infection and grazing (p-value = 0.403), (table 3.2.3)

3.2.7 Source of animals:

. Among 155 animals from white Nile, 82 were found infected .The rate of infection in white nil was (52.9%).while among one animals from kordofan these one animal was infected The rate of infection in kordofan was (100%) (table3.2.2)

The Chi-square results showed that there is no significant association between the infection and source of animal (p-value = 0.347), (table 3.2.3).

3.2.8 Water source:

Among 65 animals drinking from tap water, 39 were found infected. The rate of infection was (60%). Among 56 animals drinking from canal , 23 were infected. The rate of infection was (41%). Among these 35 animals drinking from river, 21 were found infected. The rate of infection was 60%. (table 3.2.2).

The Chi- square test showed significant association between the infection and water sours (p-value = 0.076), (table 3.2.3).

3.2.9 Presence of snails:

Among 37 animals with no presence of snails 20 animals found infected .The rate of infection with no presence of snails was (54%) . Among

119animals with presence of snails 63 animals were found infected .The rate of infection with presence of snails was (52.9%) (table 3.2.2).

The Chi- square test showed significant association between the infection and presence of snails (p-value = 0.906), (table 3.2.3)

3.10 Presence of water bodies:

Among 44 animals with no presence of water bodies 24 animals were found infected .The rate of infection with no presence of water bodies was (54.5%) . Among 111 animals with presence of water bodies 59 animals were found infected .The rate of infection with presence of water bodies was (52.6%) (table 3.2.2) .

The Chi- square test showed no significant association between the infection and presence of water bodies (p-value = 0.557), (table 3.2.3)

3.2.11: Vegetation:

Among 27 animals with no vegetation 10 animals were found infected .The rate of infection with no vegetation was (37%) . while among 129 animals with vegetation 73 animals were found infected .The rate of infection with vegetation was (56.5%)(table3.2.2).

The Chi- square test showed no significant association between the infection and vegetation (p-value = 0.064), (table 3.2.3)

3.2.12 Knowledge about disease:

Among 34 animals with no knowledge of owner about disease 18 animals were found infected .The rate of infection was (53%) .while among 122 animals with knowledge of owner about disease 65 animals were found infected .the rate of infections was (53.2%) (table3.2.2). .

The Chi- square test showed no significant association between the infection and knowledge about disease (p-value = 0.972), (table 3.2.3).

3.2.13 Manure disposal:

Among 133animals with no manure disposal 66 animals were found infected .The rate of infection with no manure disposal was (49.6%) . while among 23 animals with manure disposal 17 animals were found infected .The rate of infection with manure disposal was (74%).(table 3.2.2).

The Chi- square test showed significant association between the infection and manure disposal. (p-value = 0.031), (table 3.1.3).

3.2.14 fasciolaiasis:

Among 125 animals in negative fasciolasis 64 animals were found infected .The rate of infection with negative fasciolasis was (51.2%) . while among 31 animals in positive fasciolasis19 animals were found infected .The rate of infection with positive fasciolasis was (61.2%)(table 3.2.2).

The Chi- square test showed no significant association between the infection and fasciolasis (p-value = 0.314), (table 3.2.3).

3.2.15 schistosoma:

Among 154 animals in negative schitosomiasis 81 animals were found infected .The rate of infection with negative schitosomiasis was (52.5%) . while among 2 animals in positive schitosomiasis 2 animals were found infected .The rate of infection with positive schitosomiasis was (100%) (table 3.2.2).

The Chi- square test showed no significant association between the infection and schitosomiasis (p-value = 0.182), (table 3.2.3).

3.2.16 other diseases:

Among 146 animals in negative to other diseases examined 80 animals were found infected .The rate of infection with negative to other diseases examined was (54.7%) .while among 10 animals with positive to other diseases 3 animals were found infected .The rate of infection with positive to other diseases was (30%) (table3.2.2).

The Chi- square test showed significant association between the infection and other diseases (p-value = 0.128), (table 3.2.3).

Table 3.2.2: Summary of cross tabulation for the rate of paramphistomiasis in each category of the potential risk factors in 156 cattle from Rabak slaughterhouse examined by ELISA test

Risk factors	**No. tested**	**No. affected(%)**
Sex		
Female	122	69(56.5)
male	34	14(43.7)
Age		
Young(≤5)	83	42(50.6)
Old(>5)	73	41(56.1)

Breed		
local	88	45(51.1)
cross	68	38(55.8)
Body condition		
Poor	73	41(56.1)
Good	83	42(50.6)
Grazing type		
indoor	61	35(57.3)
outdoor	95	48(50.5)
Source		
Kordofan	1	1(100)
White Nile	155	82(52.9)
Water source		
Tap	65	39(60)
Canal	56	23(41)
River	35	21(60)

Table 3.2.2: continued :

Risk factors	No. tested	No. affected(%)
Snails presence		
No	37	20(54)
Yes	119	63(52.9)
Water bodies		
No	44	24(54.5)
Yes	112	59(52.6)

Vegetation		
No	27	10(37)
Yes	129	73(56.5)
Knowledge		
No	34	18(52.9)
Yes	122	65(53.2)
Manure disposal		
No	133	66(49.6)
Yes	23	17(74)
Fasciolaiasis		
-ve	125	64(51.2)
+ve	31	19(61.2)

Table 3.2.2: continued :

Risk factors	**No. tested**	**No. affected(%)**
Other diseases		
-ve	146	80(54.7)
+ve	10	3(30)
Schitosomaiasis		
-ve	154	81(52.5)
+ve	2	2(100)

Table 3.2.3: Summary univariate analysis for the association between paramphistomiasis and potential risk factors in 156 cattle examined at Rabak slaughterhouse by ELISA test using the Chi_square test

Risk factors	No. inspected	No. affected (%)	d.f	X^2 value	p-value
Sex					
Female	122	69(56.5)	*1*	2.725	.112
Male	34	14(43.7)			
Age (years)					
Young(≤5)	83	42(50.6)	1	.483	.487
Old(5)	73	41(56.1)			
Breed					
local	88	45(51.1)	1	.347	.556
cross	68	38(55.8)			
Body condition			1	.483	.487
Poor	73	41(56.1)			
Good	83	42(50.6)			
Grazing type			1	.700	.403
indoor	61	35(57.3)			
outdoor	95	48(50.5)			

Source			1	.585	.347
Kordofan	1	1(100)			
White Nile	155	82(52.9)			
Water source			2	5.166	.076
Tap	65	39(60)			
Canal	56	23(41)			
River	35	21(60)			

Table 3.2.3 continued :

Risk factors	**No. tested**	**No. affected (%)**	**d.f**	**X^2 value**	**p- value**
Snails presence			1	.014	.906
No	37	20(54)			
Yes	119	63(52.9)			
Water bodies			2	1.169	.557
No	44	24(54.5)			
Yes	112	59(52.6)			
Vegetation			1	3.428	.064
No	27	10(37)			
Yes	129	73(56.5)			
Knowledge			1	.001	.972
No	34	18(52.9)			
Yes	122	65(53.2)			
Manure disposal	133	66(49.6)	1	4.646	.031
No	23	17(74)			
Yes					
Fasciolaiasis			1	1.016	.314
-ve	125	64(51.2)			
+ve	31	19(61.2)			

Schitosomaiasis			1	1.782	.182
-ve	154	81(52.5)			
+ve	2	2(100)			

Table 3.2.3 continued :

Risk factors	No tested	No affected (%)	d.f	Chi-square value	p- value
Other diseases			1	2.311	.128
-ve	146	80(54.7)			
+ve	10	3(30)			

Table 3.2.4 multivariate analysis for The association between paramphistomiasis (diagnosed by ELISA test)and potential risk factors in 156 cattle examined at Rabak slaughterhouse

Risk factors	No. inspected	No. affected (%)	Exp(B)	P-value	95% CI for EXP (B)	
					LOWER	UPPER
Sex						
Female	122	69(56.5)	2.098	.112	.890	4.944
male	34	14(43.7)	Ref			
Water sours						
Tap	65	39(60)	.014	.076		
Canal	56	23(41)	Ref			
River	35	21(60)	.055	.023	.148	1.020
Vegetation						
No	27	10(37)	Ref			
Yes	129	73(56.5)	.432	.064	.131	.896
Manure disposal						
No	133	66(49.6)	Ref			
Yes	23	17(73.9)	.477	.031	.159	1.429
Schitosomiasis						
-ve	154	81(52.5)	Ref			
+ve	2	2(100)	.000	.182	.000	
Other diseases						
-ve	146	80(54.7)	3.788	.128	.743	19.320
+ve	10	3(30)	Ref			

Chapter Four
Discussion

Results of the present study have increased knowledge on the epidemiology of paramphistomiasis in cattle in rabak slaughterhouse in White Nile state of the Sudan, investigated by using fecal sedimentation test, ELISA technique and questionnaires. Fecal sedimentation showed that the sero-prevalence rate of paramphistomiasis was considerably high in the study area. While few studies have been conducted on paramphistomiasis in cattle in the Sudan, which, did not include investigations on the potential risk factors contributing to the occurrence and spread of paramphistomiasis among cattle populations.

Therefore, this study was conducted to estimate the sero-prevalence rate of paramphistomiasis in cattle and to investigate potential risk factors associated with the occurrence of paramphistomiasis in white Nile state.

In this study, the overall sero-prevalence rate of egg of paramphistomiasis in cattle fecal samples collected from Rabak slaughterhouse in White Nil state were found to be 29.5% (46/156) by fecal sedimentation test.

The results obtained from fecal sedimentation in the present study was higher than the sero prevalence reported by *GadAlkareem* et al (2012) in sudan who reported a sero prevalence of 11.25% (18/160) in cattle, by *Sanchís* et al

(2013) in spain who reported a seroprevalence of 7% (803/56) ,by Krishna et al(2013) in Bangladesh who reported a seroprevalence of 30% (107/32) , by *Shabih* et al(2006) in India**,** who reported a sero prevalence of 1.99% (351/7) , by P. *Dı´az,et al*.(2006) in spain who reported a seroprevalence of 10.1% (524/53), by *khan et al* (2008) in India who reported a seroprevalence of 7.83% (2400/188), and by Shabih *et al*(2006) in India who reported a seroprevalence of 3.4% (651/22). however the sero prevalence reported in the present study was lower than the seroprevalences reported in bangladesh of 53.1% (360/191) by paul *et al* (2011). In Ethiopia of 45.8%(384/176) by Yenenehet *al* (2012). and in Ethiopia of 44.23% (104/46) by fromsa *et al* (2011). This could be explained by the differences in the tested sample size (n), practicing of traditional communal grazing and geographical regions.

The overall sero-prevalence rate of antibodies against paraphistomiasis in cattle serum samples collected from Rabak slaughterhouse in white nil state were found to be 53.2% (83/156) by ELISA . However, this finding was higher than the seroprevalence reported by Sanchís *et al* (2013) in spain who reported a seroprevalence of 29% (803/232). While on the other hand, the seroprevalence reported by ELISA in this study was lower than the seroprevalence reported by P. Dı´az,*et al*.(2006) in Spain who reported a sero prevalence of 61.2% (524/321). This difference could be

elaborated by the differences in the tested sample size (n), animal production systems and geographical regions.

Knowledge of risk factors associated with paraphistomiasis in cattle is an important pre-requisite for the design and implementation of effective control strategies and for management programs that can lead to the control and eradication of the disease. knowledge of these risk factors and their association and contributions to the occurrence and spreading of paraphistomiasis among cattle populations also is a good aid for clinical diagnosis and for determining the epidemiology and patterns of the disease. very Few studies in the Sudan have addressed risk factors associated with sero-positivity to paraphistomiasis in cattle .

In the current study, univariate analysis using Chi -square, with a confidence interval of 95% at a *p-value* of ≤0.25 was used to identify potential risk factors associated with fecal sedimentation test positivity for paraphistomiasis infection in cattle. Significant risk factors associated with being fecal sedimentation test positive in the univariate analysis were found to be Breed ($X2$ = 4.437, p = 0.035), Body condition ($X2$ = 6.918, p = 0.009), Grazing type ($X2$ =6.367 , p = 0.012), Snail presence ($X2$ =10.6 , p = 0.001), Water bodies ($X2$ = 2.934, p = 0.231),Knowledge of owner about disease ($X2$ = 1.656, p = 0.198), Manure disposal ($X2$ = 3.508, p = 0.06),and Other disease ($X2$ = 4.468, p = 0.035).

The positive association of Breed with fecal sedimentation test paramphistomiasis-positivity in cattle is in agreement with the findings of Yeneneh *et al* (2012), and Krishna *et al* (2013). The positive association of Sex with fecal sedimentation test paramphistomiasis-positivity in cattle is in agreement with the findings of Krishna *et al* (2013), whilst the positive association of Body condition, Grazing type, Snail presence, Water bodies, Knowledge of owner about disease, Manure disposal, and Other disease. with fecal sedimentation test paramphistomiasis-positivity in cattle are investigated for the first time . This positive association of breed as risk factor could be explained by the fact that local breed is known for its tolerance to parasitic diseases.

In the current study, univariate analysis using Chi -square, with aconfidence interval of 95% at a *p-value* of ≤0.25 was used to identify potential risk factors associated with ELISA-positivity for paramphistomiasis infection in cattle. Significant risk factors associated with being ELISA positive in the univariate analysis were found to be Sex (*X2* = 2.725, p = 0.112), water source(*X2* = 5.166, p = 0.076),vegetation(*X2* = .428, p = 0.064), manure disposal (*X2* = 4.646, p = 0.031) shitiosomaiasis (*X2* = 1.782, p = 0.182),and other diseases (*X2* = 2.311, p = 0.128), the positive association of sex, water source, vegetation, manure disposal, shitiosomaiasis, and other diseases with ELISA test

paramphistomiasis-positivity in cattle are investigated for the first time.

The multivariate analysis, using logistic regression, with a confidence interval of 95% and a *p- value* of ≤0.05 was used to assess the association between identified significant risk factors in the univariate analysis in combination towards a positive fecal sedimentation test status for paramphistomiasis in cattle. However, some potential risk factors which were regarded to be important with p ≤ 0.25 in the univariate analysis were also entered into the multivariate analysis. This analysis showed an association between being fecal sedimentation test positive status for paramphistomiasis infection in cattle and breed(Exp (B) = . 565), body condition (Exp (B) = .1.5), grazing type (Exp (B) = 2.07), snail presence (Exp (B) = .092), other disease (Exp (B) = 2.17), The positive association of body condition with fecal sedimentation test paramphistomiasis-positivity in cattle is in agreement with the findings by fromsa *et al* (2011)Whilst the positive association of breed, grazing type, snail presence, and other disease with fecal sedimentation test paramphistomiasis-positivity in cattle are investigated for the first time. This positive association of body condition as risk factor could be explained by the fact that the fluke causes high protein loses in ruminant also the emaciated animal have lower resistance to fluke than cattle with a good body condition.

The multivariate analysis, using logistic regression, with a confidence interval of 95% and a *p- value* of ≤0.05 was used to assess the association between identified significant risk factors in the univariate analysis in combination towards a positive ELISA status for paramphistomiasis in cattle However, some potential risk factors thought to be important with p ≤ 0.25 in the univariate analysis were also entered into the multivariate Analysis. This analysis showed an association between being ELISA test positive status for paramphistomiasis infection in cattle and water source. It showed that cattle witch drink from river (Exp (B) = . 1.365),, and manure disposal. (Exp (B) = .477), The positive association of water source and manure disposal with ELISA test paramphistomiasis in cattle were investigated for the first time.

Conclusion:

From the results of the study, it can be concluded that cattle parampistomiasis according to serological diagnosis is prevailing in Rabak slaughterhouse of White Nile state at high sero-prevalence rate by ELISA test (53.2%). Compare to the much lower prevelance by fecal sedimentation test (29.5%).

Based on the results of the study, the risk factors associated with paramphistomiasis in cattle in Rabsk slaughterhouse of White Nile state were: breed, grazing type ,body condition, water source, snail presence, water bodies, knowledge of owner about disease, manure disposal and other disease with fecal sedimentation test and sex, water source, vegetation, manure disposal, *Schistosomaiasis* and other disease with ELISA test .

Recommendations:

The study shows the need for:

1- More studies on potential risk factors that enhance the spread and
transmission of paramphistomiasis in cattle in the Sudan.

2- Enforcement of legislation that will put end to backyard and road side slaughtering practices

3- Extension and communication programs should be implemented to enable sheep and other livestock owners to understand the importance of the disease.

4- Integrated control and eradication program should immediately be launched as recommended by OIE.

5- The scheme of initiation of a regional network for surveillance, control and eradication of this important disease in the surrounding Africa countries

Referance

Abrous, m. ; Rondeloaud, d. and Drefuss, G. (2000) . cercarialp roductivity of radial generation in single-miracidum infection of lymenaea truncutula with paramphistomum daubneyi or fasciola hepatica **.** *journal of helemінthology,* 74 , pp: 1-5.

Abrous, D. ; Rondelaud, S. and Dreyfuss, G. (2000). A field study of natural infections in three freshwater snails with Fasciola hepatica and/or Paramphistomum daubneyi in central France. *Journal of Helminthology.* 74 , PP:189-194

Abunza, M.D. ; Ahmad, A. and Afana, S. (2008). Prevalence and Paramphistomiasis in Ruminants Slaughtered at Sokoto Central Abattoir Nigerian *Journal of Basic and Applied Sciences* vol. 16.pp: 287 - 292

Adejoju, O.A. ; Bamidele, A.A. ; and Olakunle, B.A

(2008) . Acomparative study of three methods for detecting Fasciola infections in Nigerian cattle. Veterinary skiarhiV 78 (5), 411-416

Boray, J. (1959). "Studies on intestinal amphistomosis in cattle". *The Australian Veterinary Journal*, 35 (6), pp:282–287.

Brown, D.S. (2005). Freshwater Snails Of Africa And Their Medical Importance (2 ed.). *Taylor & Francis Ltd*. Pp:366–370.

Chai, J.Y. ; Shin, E.H. ; Lee, S.H. and Rim, H.J. (2009). "Foodborne intestinal flukes in Southeast Asia" .*The Korean Journal of Parasitology*, 47, pp:69–102.

Chingwena, G. ; Mukaratirwa, S. ; Chimbari, M. ; Kristensen, T.K. and Madsen, H. (2000). Population dynamics and ecology of freshwater gastropods in the highveld and lowveld regions of Zimbabwe, with emphasis on schistosome and amphistome intermediate hosts**.** *African Zoology*, 39(1), pp:55–62.

Cringoli, G. ; Taddei, R. ; Rinaldi, L. ; Veneziano, V. ; Musella, V. ; Cascone, C. ; Sibilio, G. and Malone, J.B. (2004). Use of remote sensing and geographical information systems to identify environmental features that influence the distribution of paramphistomosis in sheep from the southern Italian Apennines**.** *Veterinary Parasitology*, 122, pp: 15–26.

Dı´az, P. ; Lomba, C. ; Pedreira, J. ; Arias, M. ; Sa´nchez, A.R. ; Sua´rez, J. ;Dı´ez, B.P. ; Morrondo, P. and Paz-Silva A. (2006). Analysis of the IgG antibody response against Paramphistomidae trematoda in naturally infected cattle Application to serological surveys. *Veterinary Parasitology*, 140, pp:281–288.

Eslami, A. ; Halajian, A. ; and Bokaie, S. (2012). A survey on the bovine amphistomiasis in Mazanderan province, north of Iran. Iranian *Journal of Veterinary Research*, 12 , pp:34-38

Estuningsih, E.S. ; Widjanti, S. ; Adiwinata, G. and Piedrafita D.(2004). Detection of coproantigens by sandwich ELISA in sheep experimentally infected with Fasciola gigantica. Tropical Biomedicine, 21, pp: 51–56.

FAO (2009), Assessment mission report, Crop production and food security assessment for the northern states of the Sudan,

Foster, A.P. ; Otter, A. ; O'Sullivan, T. ; Cranwell, M.P. ; Twomey, D.F. ; Millar, M.F. and Taylor, M.A. (2008). Rumen fluke (paramphistomosis) in British cattle. *Veterinary Record*, 162, pp:528.

Fromsa, A. ; Meharenet, B. ; and Mekibib, B. (2011). Major trematode infection of cattle slaughtered at Jimma Municipality abattoir and the occurrence of intermediate hosts in selected water bodies of the zone. *Animal and veterinary advances*, 10(12), pp:1592-1597

Gad Alkareem, I. ; Abdelgadir, A. and Elmalik, K. (2012). Study on prevalence of parasitic diseases in cattle in Abyei area Sudan**.** *Journal of Cell and Animal Biology*, 6(6), pp:88-98.

Horak, I.G. (1967). Host-parasite relationships of Paramphistomum microbothrium Fischoeder, 1901, in experimentally infested ruminants, with particular reference to sheep. Onderstepoort Journal of Veterinary Research, 34, pp:451-540.

Kaur, S. ; Singla, L.D. ; Hassan, S.S. and Juyal, P.D. (2009). Standardization and application of indirect plate ELISA for

immunodiagnosis of paramphistomosis in ruminants. *journal parasitic diseas*, 33(1-2), pp:70-76.

Khan, U. J. Tanveer, A. ; Maqbool, A. and Masood, S. (2008). Epidemiological studies of paramphistomosis in cattle. *Veterinarski Arhiv*, 78 (3), pp: 243-251.

Kiusluka, L. and cambarage, D. (1996). Disease of small ruminant in sum_suhran Africa. edited and published by vetaid center for tropical *veterinary medicine*. pp:17.

Krishna, K. G. ; Jahan, T. M. ; Jalal, M. S. ; and Sirajul, I.M. (2013). Study on Paramphistomiosis in Cattle at Sonatala Upazila, Bogra, Bangladesh. *Journal of Advances in Parasitology*. vol 1(1), pp:4 – 5

Lowry, O. H., N. J. Roerbrough, A. L. Farr, and Randal. J. R. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265–272.

Magea, C. ; Bourgneb , H. ; Toullieub, J. ; Rondelaud, D. and Dreyfuss, G. (2002). Fasciola hepatica and Paramphistomum daubneyi changes in prevalences of natural infections in cattle and in Lymnaea truncatula from central France over the past 12 years **.** *vet.res*, 33 , pp:439-447.

Martin, S.W., Meek, A.H and Welleberg, P., (1988), *Veterinary epidemiology*/ principles and Methods, **2** : 30-31.

Mavenyengwa, M. ; Mukaratirwa, S. ; Obwolom ; and Monrad, J. (2006). Observations on mass production of Calicophoron microbothrium

metacercariae from experimentally and naturally infected Bulinus tropicus**.** Onderstepoort *Journal of Veterinary Research*, 73, pp:95–100**.**

Melaku, S. and Addis, M. (2012). Prevalence and Intensity of Paramphistomum in Ruminants Slaughtered at Debre Zeit Industrial Abattoir, Ethiopia. *Global Veterinaria*, 8(3), pp:315-319.

Mogdy, H. ; Al-Gaabary, T. ; Salama, A. ; Osman, A. and Amera, G. (2009). Studies on Paraphisstomiasis in Ruminants in Kafrelsheikh. *Journal of Veterinary Medicine*, 10, pp: 116-136.

Mukhtar,O.O.(2010). Studies on the epidemiology. Immunodignosis and the chemotherapy of fasciola gigantica in the white Nile state, sudan . University of Khartoum. Sudan

Musa, A. I. ; Shiwei, X. U. ; and Wen, I. U. (2013). The Impact of Social Factors of Rural Households on Livestock Production and Rural Household Income in White Nile State of Sudan. International *Journal of Agricultural and Food Research*. Vol. 2 (4), pp: 1-13

NBHS (2009), Sudan National Baseline Household Survey, North Sudan - Tabulation Report. Sudan Central Bureau of Statistics, Kosti department, White Nile state, Khartoum, Sudan

NSW DPI.(2007) Stomach flukes (paramphistomes) in ruminants www.dpi.nsw.gov.au

Olsen, O.W. (1974). Animal Parasites: Their Life Cycles and Ecology (3 ed.). Dover Publications, Inc., New York/University Park Press, Baltimore, US. pp: 273–276.

Pacenovský, J. ; Záhor, Z. and Krupicer, I. (1987). The first finding of Paramphistomum daubneyi in beef cattle in Algeria. , 32(6), pp:379-84.

parasites of the livestock (www.parasitepedia.net). 2013.

Paul, A. K. ; Talukder, M. ; Begum, K. and Rahman M. A.(2011). Epidemiological investigation of Paramphistomiasis in cattle at selected areas of Sirajgonj district of Bangladesh. *Journal. Bengladesh Agri university* 9(2):229-232

Prevalence and of Paramphistomiasis in Ruminants Slaughtered at Sokoto Central Abattoir. (2008). Nigerian Journal of Basic and Applied Sciences, 16, pp:287 – 292.

Raza, M. A. ; Murtaza, S. ; Bachaya, H. A. and Hussain, A. (2009). Prevalence of Paramphistomum cervi in Ruminants Slaughtered in District Muzaffar Garh. *Pakistan Vet. J.*, 29(4), pp: 214-215.

Rinaldi, L. ; Perugini, A. ; Capuano, F. ; Fenizia, D. ; Musella, V. ; Veneziano , V. and Cringoli, G. (2005) . Characterization of the second internal transcribed spacer of ribosomal DNA of Calicophoron Daubneyi(paramphistoma) from various hosts and locations in southern Italy**.** *Veterinary Parasitology*, 131, pp: 247–253.

SANABRIA, R.E. and ROMERO, J.R. (2008). Review and update of paramphistomosis. *HELMINTHOLOGIA*, 4(2) , pp:64 – 68.

Sanchísb, J. ; Sánchez ,R. ; Macchib, M.I. ; Pineiroa, P. ; Suáreza, J.L. ; Cazapal, C. ; Maldinib, G. ; Venzalb, J.M. ; Silvaa, A. and Ariasa, M.S.

(2013). Infection by Paramphistomidae trematodes in cattle from two agricultural regions in NW Uruguay and NW Spain. *Veterinary Parasitology*, 191, pp: 165– 171.

Shabih, H. and Juyal, P. (2006). Towards Epidemiology Of Paramphistomosis In Domestic Ruminants In Punjab. *Indian Journal of Animal Sciences*, 42 (4), PP: 272-282.

Shabih, H.S. and Juyal, P.D. (2006). Diagnosis of Paramphistomosis in Domestic Ruminants in Punjab (INDIA). *Veterinary Epidemiology and Economics Available*

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. Bailliere, Tindall, London, pp:809.

Uddin, M. Z. ; Farjana, T. ; Begum, N. and Mondal, M. M. (2006). Prevalence of Amphistomes in Black Bengal Goats in Mymensingh District. Bangl. *J.Vet. Med*. 4(2), pp:103–106.

Waal, T..D. (2011). paramphistomum a brief review. *Irish Veterinary Journal*, 63, pp:5.

Wael, M. ; Sara, V. ; Karam, I. Devkota, R. ; Gerald M. and Eric S. (2010). A molecular approach for identification of paramphistomes from Africa and Asia**.** *Veterinary Parasitology*, 174 , pp: 234–240.

Warleta, M. G. ; Lladosa, S. ; Hermida , J. C. ; Ibeas A. ; Conesa, D. ; Mu˜noz, F. ; Qu´ılez, A. ; Gonz´alez, Y. and Mezo, M. (2012). Bovine paramphistomosis in Galicia (Spain): Prevalence, intensity, aetiology and geospatial distribution of the infection.*Veterinary Parasitology*, pp: 304-4017.

Yeneneh, A. ; Kebede, H. ; Fentahun ,T. and Chanie ,M. (2012). Prevalence of cattle flukes infection at Andassa Livestock Research Center in north-west of Ethiopia.*Veterinary Research Forum*, 3(2), pp:85- 89

Appendix 1

1: Frequency for distribution of paramphistomasis in 156 cattle examined by Fecal sedimentation test and ELISA at Rabak slaughterhouse according to potential risk factors investigated :

Table 1.1: Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	Female	122	78.2	78.2	78.2
	Male	34	21.8	21.8	100.0
	Total	156	100.0	100.0	

Table 1.2: Age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	Young	83	53.2	53.2	53.2
	Old	73	46.8	46.8	100.0
	Total	156	100.0	100.0	

Table 1.3: Breed

	Frequency	Percent	Valid Percent	Cumulative Percent

Valid				
Local	88	56.4	56.4	56.4
Cross	68	43.6	43.6	
Total	156	100.0	100.0	100.0

Table 1.4 Body condition:

	Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid				
Poor	73	46.8	46.8	46.8
Good	83	53.2	53.2	100.0
Total	156	100.0	100.0	

Table 1.5 Grazing :

	Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid				
Indoor	61	39.1	39.1	39.1
Outdoor	95	60.9	60.9	100.0
Total	156	100.0	100.0	

Table 1.6 Source of animals:

	Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**

Valid				
Kordufan	1	.6	.6	.6
W.N	155	99.4	99.4	100.0
Total	156	100.0	100.0	

Table 1.7: Water source:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
Tap	65	41.7	41.7	41.7
Canal	56	35.9	35.9	77.6
River	35	22.4	22.4	100.0
Total	156	100.0	100.0	

Table 1.8 Snail presence :

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
No	37	23.7	23.7	23.7
Yes	119	76.3	76.3	100.0
Total	156	100.0	100.0	

Table 1.9: Water bodies :

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
No	44	28.2	28.2	28.2
Yes	111	71.2	71.8	99.4
Total	156	100.0	100.0	100.0

Table 1.10 Vegetation :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	No	27	17.3	17.3	17.3
	Yes	129	82.7	82.7	100.0
	Total	156	100.0	100.0	

Table 1.11 Knowledge of owner about disease :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	No	34	21.8	21.8	21.8
	Yes	122	78.2	78.2	100.0
	Total	156	100.0	100.0	

Table 1.12 Manure disposal :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid					
	No	133	85.3	85.3	85.3
	Yes	23	14.7	14.7	100.0
	Total	156	100.0	100.0	

Table 1.13 Fasciolasis :

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
Negative	125	80.1	80.1	80.1
Positive	31	19.9	19.9	100.0
Total	156	100.0	100.0	

Table 1.14 Schitiosomaiasis :

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
		98.7	98.7	98.7
Negative	144	1.3	1.3	100.0
Positive	2			
Total		100.0	100.0	

Table 1.15 : Other disases:

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid				
Negative	146	93.6	93.6	93.6
Positive	10	6.4	6.4	100.0
Total	156	100.0	100.0	

Appendix 2

2 :Cross-tabulation tables for distribution of 156 cattle paramphistomasis examined by Fecal sedimentation test at Rabak slaughterhouse according to potential risk factors investigated :

Table 2.1 : : Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to sex :

	Sex of animal	

	Female	Male	Total
Result **-ve**	85	25	110
Total **%**	85/122X100 69.6%	25/34X100 73.5%	110/156X100 70.5%
+ve	37	9	46
Total **%**	37/122X100 30.3%	9/34X100 26.4%	46/156X100 29.4%
Total %	122 100.0%	34 100.0%	156 100.0%

Table 2.2 : : Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Age :

	age of animal		Total
	Young	Old	
Fecal			
-ve	56	54	110
Total %	56/83X100 67.4%	54/73X100 73.9%	110/156X100 70.5%
+ve	27	19	46
Total %	27/83X100 32.5%	19/73X100 26%	46/156X100 29.4%
Total %	83 100.0%	73 100.0%	156 100.0%

Table 2.3 : Distribution and prevalence of paramphistomasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Breed :

		Breed		Total
		Local	**Cross**	
Fecal	-ve	68 68/88X100	42 42/68X100	110 110/156X100
	Total %	77.2%	61.7%	70.5%
	+ve	20 20/88X100	26 26/68X100	46 46/156X100
	Total %	22.7%	38.2%	29.4%
	Total %	88 100.0%	68 100.0%	156 100.0%

Table 2.4: : Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Body condition :

		Body condition		Total
		Boor	**Good**	
Fecal	-ve	44 44/73X100	66 66/83X100	110 110/156X100
	Total %	6.2%	79.5%	70.5%
	+ve	29 29/73X100	17 17/83X100	46 46/156X100
	Total %	39.7%	20.4%	29.4%
Total %		73 100.0%	83 100.0%	156 100.0%

Table 2.5: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Grazing :

		Grazing		Total
		indoor	Outdoor	
Fecal	-ve	36 36/61X100	74 74/95X100	110 110/156X100
	Total %	59%	77.8%	70.5%

+ve Total %	25 25/61X100 40.9%	21 21/95X100 22.1%	46 46/156X100 29.4%
Total %	61 100.0%	95 100.0%	156 100.0%

Table 2.6: : Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Source :

	Sours		**Total**
	Kordofan	**W.N**	
Fecal -ve Total %	1 1/1X100 100%	109 109/155X100 70.3%	110 110/156X100 70.5%
+ve Total %	0 0/1X100 0%	46 46/155X100 29.6%	46 46/156X100 29.4%

Table 2.7: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Water source :

	Water sours			**Total**
	Tap	**Canal**	**River**	
Fecal -ve Total %	43 43/65X100 66.1%	46 46/56X100 82.1%	21 21/35X100 60%	110 110/156X100 70.5%
+ve Total %	22 22/65X100 33.8%	10 10/56X100 17.8%	14 14/35X100 40%	46 46/156X100 29.4%

Total %	65 100.0%	56 100.0%	35 100.0%	156 100.0%

Table 2.8: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Snail presence:

		Snail presence		**Total**
		No	**Yes**	
Fecal	-ve	34 34/37X100	76 76/119X100	110 110/156X100
	Total %	91.8%	63.8%	70.5%
	+ve	3 3/37X100	43 43/119X100	46 46/156X100
	Total %	8.1%	36.1%	29.4%
Total %		37 100.0%	119 100.0%	156 100.0%

Table 2.9: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Water bodies :

		Water bodies		**Total**
		No	Yes	
Fecal	-ve	35 35/44X100	74 74/111X100	110 110/156X100
	Total %	79.5%	66.6%	70.5%
	+ve	9 9/44X100	37 37/111X100	46 46/156X100
	Total %	20.4%	33.3%	29.4%

Table 2.10: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Vegetation:

		Vegetation		Total
		No	**Yes**	
Fecal	-ve	20 20/27X100	90 90/129X100	110 110/156X100
	Total %	74%	69.7%	70.5%
	+ve	7 7/27X100	39 39/129X100	46 46/156X100
	Total %	29.5%	30.2%	29.4%

Table 2.11: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Knowledge of owner about disease :

		Knowledge		Total
		No	**Yes**	
Fecal	-ve	27 27/34X100	83 83/122X100	110 110/156X100
	Total %	79.4%	68%	70.5%
	+ve	7 7/34X100	39 39/122X100	46 46/156X100
	Total %	20.5%	31.9%	29.4%
		34	122	156
	Total %	100.0%	100.0%	100.0%

Table 2.12: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Manure disposal :

		Manure disposal		**Total**
		No	**Yes**	
Fecal	-ve	90 90/133x100	20 20/23x100	110 110/156X100
	Total %	67.6%	86.9%	70.5%
	+ve	43 43/133x100	3 3/23x100	46 46/156X100
	Total %	32.3%	13%	29.4%
Total %		133 100.0%	23 100.0%	156 100.0%

Table 2.13: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal sedimentation test at Rabak slaughterhouse related to Fasciolaiasis :

		Fasciolaiasis		**Total**
		Posative	Negative	
Fecal	-ve	88 88/125X100	22 22/31X100	110 110/156X100
	Total %	70.4%	70.9%	70.5%
	+ve	37 37/125X100	9 9/31X100	46 46/156X100
	Total %	29.6%	29%	29.4%

Table 2.14: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal at Rabak slaughterhouse related to Shistiosoma:

		Schistiosomaiasis		Total
		Posative	**Negative**	
Fecal	-ve	108 108/154x100	2 2/2x100	110 110/156X100
	Total %	70.1%	100%	70.5%
	+ve	46 46/154x100	0 0/2x100	46 46/156X100
	Total %	29.8%	0%	29.4%
Total %		154 100.0%	2 100.0%	156 100.0%

Table 2.15: Distribution and prevalence of paramphistomiasis in 156 cattle examined by fecal at Rabak slaughterhouse related to other diseases :

		other diseases		Total
		Posative	Negative	
Fecal	-ve	100 100/146x100	10 10/10x100	110 110/156X100
	Total %	68.4%	100%	70.5%
	+ve	46 46/146x100	0 0/10x100	46 46/156X100
	Total %	31.5%	0%	29.4%
Total %		146 100.0%	10 100.0%	156 100.0%

Appendix 3

3. Association between paramphistomasis infection examined by fecal sedimentation test and potential risk factors using the Chi- square test:

Table 3.1 : Association between paramphistomiasis and sex :

	Value	**Df**	**Asymp.sig. (2-sided)**
Pearson chi- square	.190	1	.663
Continuity	.050	1	.823
Likelihood Ratio	.193	1	.660
No of valid Cases	156		

Table 3.2 : Association between paramphistomiasis and age :

	Value	**Df**	**Asymp.sig. (2-sided)**
Pearson chi- square	.790	1	.374
Continuity	.508	1	.476
Likelihood Ratio	.793	1	.373
No of valid Cases	156		

Table 3.3 : Association between paramphistomiasis and breed :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	4.437	1	.035
Continuity		1	.054
Likelihood Ratio	3.722	1	.036
No of valid Cases			
	4.418		
	156		

Table 3.4 : Association between paramphistomiasis and body condition :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	6.918	1	.009
Continuity	6.023	1	
Likelihood Ratio		1	.014
No of valid Cases	6.955		
	156		.008

Table 3.5 : Association between paramphistomiasis and grazing :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	6.367	1	.012
Continuity	5.492	1	.012
Likelihood Ratio	6.280	1	.019
No of valid Cases	156		

Table 3.6 : Association between paramphistomiasis and source of animal :

	Value	Df	Asymp.sig. (2-sided)

Pearson chi- square	.421	1	.516
Continuity	.000	1	1.000
Likelihood Ratio	.701	1	.402
No of valid Cases	156		

Table 3.7 : Association between paramphistomiasis and water source :

	Value	**Df**	**Asymp.sig. (2-sided)**
Pearson chi- square	6.097	2	.516
Continuity			
Likelihood Ratio	6.350	2	1.000
No of valid Cases	156		

Table 3.8: Association between paramphistomiasis and snail presence :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	10.662	1	.001
Continuity	9.357	1	.002
Likelihood Ratio	12.694	1	.000
No of valid Cases	156		

Table 3.9: Association between paramphistomiasis and water bodies :

	Value	**Df**	**Asymp.sig.** (2-sided)

Pearson chi- square	2.934	1	.231
Continuity	3.324	1	
Likelihood Ratio		1	.190
No of valid Cases	156		

Table 3.10: Association between paramphistomiasis and vegetation :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.199	1	.655
Continuity	.046	1	.830
Likelihood Ratio	.203	1	.652
No of valid Cases	156		

Table 3.11: Association between paramphistomiasis and knowledge of owner about disease :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	1.656a	1	.198
Continuity	1.154	1	.283
Likelihood Ratio	1.744	1	.187
No of valid Cases	156		

Table 3.12: Association between paramphistomiasis and manure disposal :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	3.508	1	.061
Continuity	2.642	1	.104
Likelihood Ratio	3.999	1	.046
No of valid Cases	156		

Table 3.13: Association between paramphistomiasis and fasciolaiasis :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	.004	1	.951
Continuity	.000	1	1.000
Likelihood Ratio	.004	1	.950
No of valid Cases	156		

Table 3.14: Association between paramphistomiasis and shistiosomaiasis :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	.847	1	.357
Continuity	.020	1	.889
Likelihood Ratio	1.408	1	.235
No of valid Cases	156		

Table 3.15: Association between paramphistomiasis and other diseases :

	Value	Df	Asymp.sig. (2-sided)

Pearson chi- square	4.468	1	.035
Continuity	3.081	1	.079
Likelihood Ratio	7.270	1	.007
No of valid Cases	156		

Appendix 4

2: Cross-tabulation tables for distribution of 156 cattle paramphistomasis examined by ELISA at Rabak slaughterhouse according to potential risk factors investigated :

Table 2.1 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by Elisa at Rabak slaughterhouse related to sex :

		Sex		**Total**
		female	**Male**	
Elisa	-ve	53 53/112X100	20 20/34X100	73 73/156X100
	Total **%**	47.3%	58.8%	46.7%
	+ve	69 69/112X100	14 14/34X100	83 83/156X100
	Total **%**	61.6%	41.7%	53.2%
Total **%**		112 100.0%	34 100.0%	156 100.0%

Table 2.2 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to age :

	Age		**Total**
	young	**Old**	

Elisa -ve	41 41/83X100	32 32/73X100	73 73/156X100
Total %	49.3%	43.8%	46.7%
+ve	42 42/83X100	41 41/83X100	83 83/156X100
Total %	50.6%	56.1%	53.2%
Total %	83 100.0%	73 100.0%	156 100.0%

Table 2.3 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to breed :

	Breed		**Total**
	local	**Cross**	
Elisa -ve	43 43/88X100	30 30/68X100	73 73/156X100
Total %	48.8%	44.1%	46.7%
+ve	45 45/88X100	38 38/68X100	83 83/156X100
Total %	51.1%	55.8%	53.2%
Total %	88 100.0%	68 100.0%	156 100.0%

Table 2.4 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to body condition :

	Body condition		**Total**
	Boor	**Good**	
Elisa -ve	32 32/73X100	41 41/83X100	73 73/156X100
Total %	43.8%	49.3%	46.7%

+ve Total %	41 41/73X100 56.1%	42 42/83X100 50.6%	83 83/156X100 53.2%
Total %	73 100.0%	83 100.0%	156 100.0%

Table 2.5: Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to grazing :

		Grazing		**Total**
		Indoor	**Outdoor**	
Elisa	-ve Total %	26 26/61x100 42.6%	47 47/95x100 49.4%	73 73/156X100 46.7%
	+ve Total %	35 35/61x100 57.3%	48 48/95x100 50.5%	83 83/156X100 53.2%
Total %		61 100.0%	95 100.0%	156 100.0%

Table 2.6: Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to source of animal:

		Source		**Total**
		Kordofan	**W.N**	
Elisa	-ve Total %	0 0/1X100 0%	73 73/155X100 47%	73 73/156X100 46.7%

+ve Total %	1 1/1X100 100%	82 82/155X100 52.9%	83 83/156X100 53.2%
Total %	1 100.0%	155 100.0%	156 100.0%

Table 2.7: Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to water source :

		Water source			**Total**
		Tap	**Canal**	**River**	
Elisa	-ve	26 26/65X100 40%	33 33/35X100 94.2%	14 14/35X100 40%	73 73/156X100 46.7%
	+ve Total %	39 39/65X100 60%	23 23/56X100 41%	21 21/35X100 60%	83 83/156X100 53.2%
Total %		65 100.0%	56 100.0%	35 100.0%	156 100.0%

Table 2.8 : Distribution a nd prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to snail presence :

		Snail presence		**Total**
		No	Yes	
Elisa	-ve	17 17/37X100 45.9%	56 56/119X100 47%	73 73/156X100 46.7%
	+ve Total %	20 20/37X100 54%	63 63/119X100 52.9%	83 83/156X100 53.2%

Total %	37 100.0%	119 100.0%	156 100.0%

Table 2.9 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to water bodies :

		Water bodies		Total
		No	Yes	
Elisa	-ve	20 20/44X100	53 53/112X100	73 73/156X100
	Total %	45.4%	47.3%	46.7%
	+ve	24 24/44X100	59 59/112X100	83 83/156X100
	Total %	54.5%	52.6%	53.2%

Table 2.10 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to vegetation:

		Vegetation		**Total**
		NO	**Yes**	
Elisa	-ve	17 17/27X100	56 56/129X100	73 73/156X100
	Total %	62.9%	34.4%	46.7%
	+ve	10 10/27X100	73 73/129X100	83 83/156X100
	Total %	37%	56.5%	53.2%
Total %		27 100.0%	129 100.0%	156 100.0%

Table 2.11 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to knowledge :

		Knowledge		Total
		No	**Yes**	
Elisa	-ve	16 16/34X100	57 57/122X100	73 73/156X100
	Total %	47%	46.7%	46.7%
	+ve	18 18/34X100	65 65/122X100	83 83/156X100
	Total %	52.9%	53.2%	53.2%
Total %		34 100.0%	122 100.0%	156 100.0%

Table 2.12 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to manure disposal :

		Manure disposal		Total
		No	**Yes**	
Elisa	-ve	67 67/133x100	6 6/23X100	73 73/156X100
	Total %	50.3%	26%	46.7%
	+ve	66 66/133X100	17 17/23X100	83 83/156X100
	Total %	49.6%	73.9%	53.2%
Total %		133 100.0%	23 100.0%	156 100.0%

Table 2.13 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to fasciolaiasis :

		Fasciolaiasis		Total
		posative	Negative	
Elisa	-ve	61 61/125X100	12 12/31X100	73 73/156X100
	Total %	48.8%	38.7%	46.7%
	+ve	64 64/125X100	19 19/31X100	83 83/156X100
	Total %	51.2%	61.2%	53.2%
Total %		125 100.0%	31 100.0%	156 100.0%

Table 2.14 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to shistiosomaiasis :

		Shistiosomaiasis		Total
		posative	Negative	
Elisa	-ve	73 73/154X100	0 0/2X100	73 73/156X100
	Total %	47.4%	0%	46.7%
	+ve	81 81/154X100	2 2/2X100	83 83/156X100
	Total %	52.5%	100%	53.2%
Total %		154 100.0%	2 100.0%	156 100.0%

Table 2.13 : Distribution and prevalence of paramphistomiasis in 156 cattle examined by ELISA at Rabak slaughterhouse related to other diseases :

	Other diseases		Total
	posative	Negative	

Elisa -ve Total %	66 66/146X100 52.2%	7 7/10X100 70%	73 73/156X100 46.7%
+ve Total %	80 80/146X100 54.7%	3 3/10X100 30%	83 83/156X100 53.2%
Total %	146 100.0%	10 100.0%	156 100.0%

Appendix 5

5. Association between paramphistomasis infection examined by ELISA and potential risk factors using the Chi- square test:

Table 5.1 : Association between paramphistomiasis and sex :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	2.527	1	.112
Continuity	1.947	1	.163
Likelihood Ratio	2.527	1	.112
No of valid Cases	156		

Table 5.2 : Association between paramphistomiasis and age :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.483	1	.487
Continuity	.285	1	.593
Likelihood Ratio	.483	1	.487
No of valid Cases	156		

Table 5.3 : Association between paramphistomiasis and breed :

	Value	**Df**	**Asymp.sig. (2-sided)**
Pearson chi- square	.347	1	.556
Continuity	.183	1	.669
Likelihood Ratio	.347	1	.556
No of valid Cases	156		

Table 5.4 : Association between paramphistomiasis and body condition :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.483	1	.487
Continuity	.285	1	.593
Likelihood Ratio	.483	1	.487
No of valid Cases	156		

Table 5.5 : Association between paramphistomiasis and grazing :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.700	1	.403
Continuity	.452		.501
Likelihood Ratio		1	.402
No of valid Cases	.702		
		1	
	156		

Table 5.6 : Association between paramphistomiasis and source of animal :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.885	1	.347
Continuity	.000	1	1.000

Likelihood Ratio	1.268	1	.260
No of valid Cases	156		

Table 5.7 : Association between paramphistomiasis and water Source :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	5.166	**1**	**.076**
Continuity	5.181	**1**	
Likelihood Ratio		**1**	**.075**
No of valid Cases	156		

Table 5.8 : Association between paramphistomiasis and snail presence :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	.014	1	.906
Continuity	.000	1	1.000
Likelihood Ratio	.014	1	.906
No of valid Cases	156		

Table 5.9 : Association between paramphistomiasis and water bodies :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	1.169	1	.557
Continuity			
Likelihood Ratio	1.551	1	.461
No of valid Cases	156	1	

Table 5.10 : Association between paramphistomiasis and vegetation :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	3.428	1	.064
Continuity	2.688	1	.101
Likelihood Ratio	3.441	1	.064
No of valid Cases	156		

Table 5.11 : Association between paramphistomiasis and knowledge :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	.001	1	.972
Continuity	.000	1	1.000
Likelihood Ratio	.001	1	.972
No of valid Cases	156		

Table 5.12: Association between paramphistomiasis and manure disposal :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	4.646	1	.031
Continuity	3.722	1	.054
Likelihood Ratio	4.848	1	.028
No of valid Cases	156		

Table 5.13: Association between paramphistomiasis and fasciola :

	Value	Df	Asymp.sig. (2-sided)
Pearson chi- square	1.016	1	.314
Continuity	.651	1	.420
Likelihood Ratio	1.025	1	.311
No of valid Cases	156		

Table 5.14: Association between paramphistomiasis and shitiosoma :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	1.782	1	.182
Continuity	.387	1	.534
Likelihood Ratio	2.547	1	.111
No of valid Cases	156		

Table 5.15: Association between paramphistomiasis and other diseases :

	Value	**Df**	**Asymp.sig.** (2-sided)
Pearson chi- square	2.311	1	.128
Continuity	1.422	1	.233
Likelihood Ratio	2.349	1	.125
No of valid Cases	156		